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INFECTION OF NEWBORN SYRIAN HAMSTERS WITH THE VIRUS OF MARE ABORTION (DIMOCK AND EDWARDS)*

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As a result of their studies of several outbreaks of abortion in mares in which no cultivable etiological agent could be found, Dimock and Edwards [1-3] demonstrated the infectious nature of the epizootic and in 1932 induced abortion in mares with filtered material from an aborted fetus.

They demonstrated also that a majority of the fetuses revealed a characteristic pathological picture peculiar to epizootic outbreaks of abortion in mares in which no bacterial infection is found. Small, multiple grayish white areas of necrosis were present in the liver which histologically were seen to extend throughout the hepatic substance (Fig. 1). Dimock [3] has described acidophilic intranuclear inclusions in the hepatic cells about the periphery of these foci and in the epithelial cells of bile ducts (Fig. 5). Similar inclusions were found by him in the bronchial epithelium (Fig. 4).

As a result of their studies, Dimock and Edwards concluded that this type of epizootic abortion in mares is due to a filterable virus which induces characteristic lesions in the fetus. Thus far their attempts to induce the disease in animals other than pregnant mares have not been thoroughly successful. The only promising results followed injection of presumably infectious material, filtered and unfiltered, into pregnant guinea pigs. In several instances abortion occurred and all the fetuses were negative on bacteriological examination. No successive transfers were made, and no characteristic pathological changes were described. Because the virus of mare abortion causes a characteristic lesion in aborted foals, the presence of this lesion would serve to establish the diagnosis in the experimentally induced disease. Failure to find the lesion and lack of serial transfer leave the evidence of infection in

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guinea pigs inconclusive. The same incompleteness of evidence applies to the experimental results of Miessner and Harms [4] and of Hupbauer,[5] although the former investigators stated that they were able to induce abortion in four passages in guinea pigs.

Through the interest of Dimock and Edwards we obtained microscopical sections of tissues and pieces of fresh frozen livers from aborted foals. In the sections we found the characteristic lesions described by Dimock, Edwards and associates, both in the liver and lung.[3] Acidophilic intranuclear inclusions were a prominent feature and gave to the pathological picture a distinctiveness quite convincing of a viral etiology. In addition to the observations of Dimock and Edwards, we have found similar inclusions in nuclei of endothelial cells.

Our first experiments were concerned with attempts to infect chick embryos with bacteriologically sterile, ground liver. The results were entirely negative, as had been previous similar attempts by Dimock and Edwards.[6] In the course of these studies we inoculated with ground liver of aborted foals, pieces of human amnion grafted on the chorioallantois of chick embryos. These inoculations induced lesions in the grafts characterized by the appearance of acidophilic intranuclear inclusions in human amniotic epithelial cells, associated with necrosis, ulceration and inflammation of the epithelial membrane of the grafts. The cellular changes were characteristic and corresponded completely with those found in the hepatic and pulmonary epithelium in the aborted foals. These experiments are described in an accompanying paper.[7] We concluded that human amniotic epithelium, in contrast to the tissues of the chick embryo, is susceptible to infection with the virus of mare abortion, and that the virus is a potential infectious agent for human beings.

Our attempts to infect pregnant and newborn mice and guinea pigs failed. We then made similar trials with newborn Syrian hamsters and these, which proved to be successful, are the subject of the present report. Our evidences of infection in newborn hamsters are entirely histopathological.

Experiments

Dimock and Edwards have sent us, in a frozen state, fresh infected livers of aborted equine fetuses at varying intervals during the past year and a half. These livers have been kept in a carbon dioxide freezing unit at a temperature of approximately −78° C. and have been the sources of virus for our experiments. A fetal liver thus frozen was infective after storage for 7 months. In no instance has bacterial contamination complicated the pathogenic activity of the virus.

In our initial attempt to infect hamsters with the virus of mare abortion we tested the possibility of inducing abortion and of transmitting the infection to the embryos *in utero*. A female hamster was inoculated intraperitoneally with 0.5 cc. of a 1:10 suspension of ground liver of an infected foal. Seventy-two hours later six normal young were born, apparently at term. The mother remained well and the young developed normally without any indication of disease. It is likely that 72 hours is too short an interval for infection to become established in the mother and be transmitted to the young, should this be possible in hamsters. Owing to our success in infecting newborn hamsters and the limited number of pregnant animals at our disposal, further attempts to infect *in utero* were not attempted.

Next we attempted to infect newborn hamsters with the virus of mare abortion by inoculating both the mother and the young. A pregnant animal was inoculated intraperitoneally with 0.5 cc. of a 1:10 suspension of ground infected liver from an aborted foal. Four days later four well developed and apparently normal young were born. Hamsters normally deliver 3 to 4 days after pregnancy is detected by inspection. Eight to 14 hours after birth each newborn was given 0.1 cc. of 1:10 suspension of infected liver intraperitoneally. Seventy-two hours later one inoculated animal was missing from the litter. On the ninth day partially eaten bodies of two of them were fixed in Zenker's fluid (5 per cent acetic acid). On the twelfth day the fourth of the litter was missing. Cannibalism of mother hamsters, if their young are diseased or injured, is common. This has presented an annoying problem since the preservation of infected tissue for histological study and for transmission of virus was essential for our purposes.

Microscopical examination of sections of the dead fetuses stained with hematoxylin and eosin showed focal areas of necrosis in the liver and heart (Figs. 2 and 3). Intranuclear inclusions present in parenchymal cells of the liver and in heart-muscle cells (Figs. 6 and 8) were entirely like those found by Dimock in the livers of aborted foals. A similar experiment, without previous injection of the mother, was repeated successfully with two additional litters, the young of which were inoculated intraperitoneally 24 to 48 hours after birth with 0.1 cc. of a 1:10 suspension of ground equine liver. Death usually occurred 3 to 5 days after inoculation and microscopical lesions were found in the liver and heart.

These results indicated the susceptibility of newborn hamsters to the infection and a second serial passage of the virus was established. The liver of a newborn hamster infected with the original horse virus

was removed aseptically. Pieces of tissue were kept for histological section. The remainder of the liver was ground, suspended in saline (1:10) and inoculated intraperitoneally into 11 one-day-old hamsters. Tissues of 6 of these were fixed at intervals between 3 and 5 days. By microscopical examination infection was recognized in each case.

In like manner a third serial passage was established and lesions in the heart and liver were recognized microscopically. In spite of the fact that this virus was lost in the fourth passage, it seems evident to us that successive transmission of infection in newborn hamsters would be uniformly successful if certain precautions were observed. For example, one should determine by microscopical examination that the particular tissues used for the inoculum are well infected in order to insure an infective quantity of virus per unit volume after dilution.

Two nonpregnant adult hamsters inoculated with infected liver have shown no clinical evidence of infection but none has been sacrificed to determine the presence of microscopical foci of infection.

Evidences of Infection in Newborn Hamsters

In equine fetuses dead of infection with the virus of mare abortion, focal areas of necrosis may be found throughout the liver, and petechial hemorrhages in the heart were described by Dimock and Edwards. Histological examination of the hepatic lesions reveals the presence of characteristic acidophilic intranuclear inclusions in parenchymal, biliary and endothelial cells in and about the miliary foci of necrosis. Similar inclusions were described by Dimock [3] as occurring in bronchial epithelium, but no description of cardiac lesions has appeared. The distribution of lesions and the presence of inclusions in endothelial cells indicate a hematogenous spread of the virus in the fetus. No lesion in the placenta of mares has been described although one might suspect its existence as an immediate source of virus extending the infection to the embryo. Such a lesion might well represent a localization of virus from the blood stream of the mare.

Similar foci of necrosis are present in the livers of newborn hamsters dead of infection with the same virus (Fig. 2). Intranuclear inclusions are found quite typically in adjoining hepatic cells (Fig. 6) but we saw none in either biliary epithelium or endothelium. In the hamsters, furthermore, similar areas of necrosis are scattered through the myocardium (Fig. 3), and in and about them nuclei of heart-muscle cells contain the characteristic inclusions (Fig. 8). No lesions and no inclusions have been found in the lung.

The lesions described and accompanying the intranuclear inclusions constitute a characteristic pathological picture. In newborn hamsters

one element in the hepatic lesion is especially accentuated, namely, the occurrence of giant cells or syncytia composed of hepatic cells, in which the affected nuclei have undergone multiple division (Fig. 7). The individual nuclei contain inclusions and the mass soon undergoes hyaline necrosis and phagocytosis.

SUMMARY

The chief object of this report is to record our morphological evidence of the experimental transmission of infection by mare-abortion virus through three serial passages in newborn hamsters. Epizootics of economic importance, such as this disease causes, might possibly be controlled by means of vaccination should a suitable method be found. Experimental study in a susceptible laboratory host should facilitate the accomplishment of such an objective.

In the course of our investigations it has been found that the mare-abortion virus is infectious likewise for human amniotic epithelium and in view of this fact one must consider it a potential agent of disease in human beings.

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DESCRIPTION OF PLATE

PLATE 88

FIG. 1. Focal area of necrosis in the liver of an aborted foal infected with the virus of mare abortion. × 46. (Figs. 1, 4 and 5 are from Dimock's sections.)

FIG. 2. Two focal areas of necrosis in the liver of a newborn hamster infected with the virus of mare abortion. A mild inflammatory reaction accompanies the necrosis. × 250.

FIG. 3. Focal area of necrosis accompanied by a mild inflammatory reaction in the myocardium of a hamster infected with the virus of mare abortion. × 250.

FIG. 4. Intranuclear inclusion of the virus of mare abortion in the bronchial epithelium of an aborted foal. × 1200.

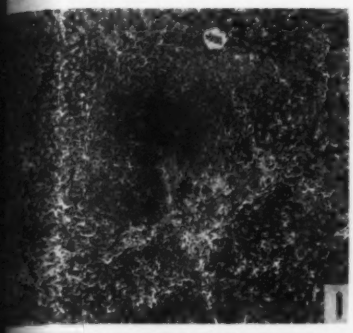
FIG. 5. Three parenchymal cells of fetal horse liver (A), showing intranuclear inclusions of mare abortion. Condensed and beaded chromatin is seen at the nuclear walls, and nuclear inclusions surrounded by clear zones. At the lower right corner is another infected parenchymal cell.

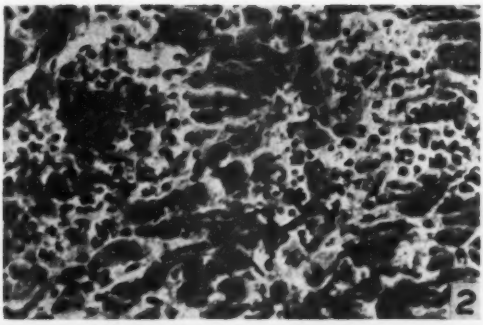
Bile duct epithelium (B), containing intranuclear inclusions of mare abortion. × 1200.

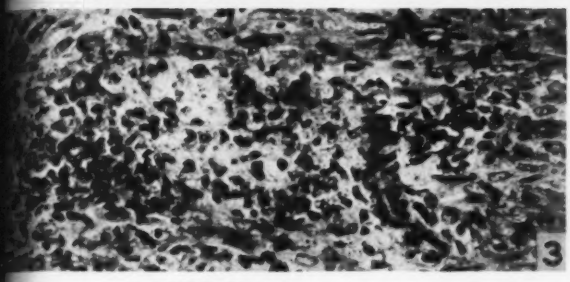
FIG. 6. Intranuclear inclusions of mare abortion in parenchymal cells of hamster liver. Three inclusions are well in focus; other cells in the area are infected. × 1100.

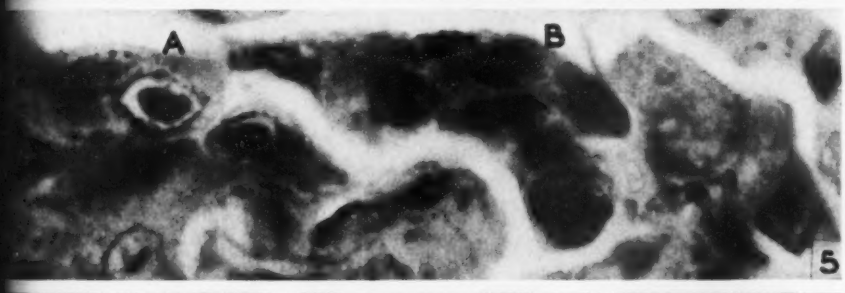
FIG. 7. Multinucleated syncytial mass in hamster liver infected with mare-abortion virus. Each nucleus contains an inclusion. There is also an infected parenchymal cell in the lower left corner. × 1100.

FIG. 8. Intranuclear inclusion of mare-abortion virus in heart-muscle cell of hamster. Three other cells in the immediate area contained inclusions not demonstrated at this focus. × 1100.





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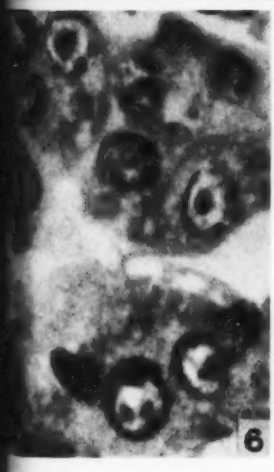

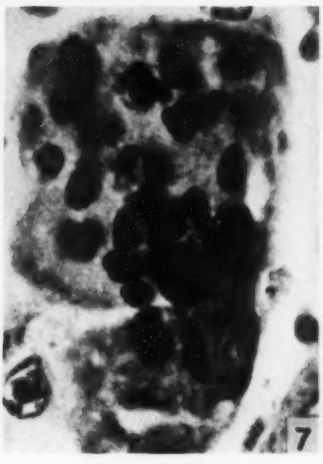

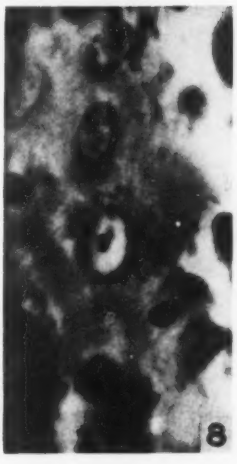

VIRUS INFECTION OF HUMAN FETAL MEMBRANES GRAFTED ON THE CHORIOALLANTOIS OF CHICK EMBRYOS*

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The possibility of successfully grafting human skin on the chorioallantois of chick embryos was demonstrated by Goodpasture, Douglas and Anderson [1] in 1938. In the experiments reported at that time mention was made of the fact that we had infected such grafts with the viruses of smallpox, vaccinia and herpes simplex. Further experiments were then carried out on the assumption that certain viruses not hitherto proven to be infectious for non-human hosts might infect a graft of human skin surviving upon chick embryonic tissue. Grafts of human skin, both from adults and, in a few instances, from children (removed for plastic operations), were inoculated with material from vesicles of the eruptions of varicella and herpes zoster, and from nodules of molluscum contagiosum. In no instance was there evidence of infection. As a result the questions naturally arose as to whether these particular skin grafts were from persons possessed of acquired immunity to the viruses employed or, if originally susceptible, whether they had become resistant because of an influence from the non-susceptible chick embryo. It was possible to submit both of these questions to experimental test and to obtain at least a partial answer, as will be described.

Our lack of success in obtaining adequate evidence of infection of human skin with vesicular material from lesions of varicella and herpes zoster led to the use of human embryonic tissue, namely, amnion and chorion, for similar experiments. Human placentas were received directly from the delivery room; the fetal membranes from an area near the placental attachment, where they were thin and semitranslucent, were stripped apart and each was spread out with the epithelial surface upward. Grafts about 1 cm. square were placed upon the chorioallantois with the epithelial surface exposed. These grafts became attached readily. The amniotic grafts were successfully inoculated with the viruses of herpes simplex, vaccinia and smallpox but in no instance did evidence of infection result from the use as inocula of vesicular contents and ground vesicular epithelium from the lesions of varicella and herpes zoster.

At the time we were also attempting to find a susceptible host for

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the virus of mare abortion which results in considerable loss to stock breeders.[2] Inoculation of chick embryos and their membranes had not been successful and it appeared that the tissues of the developing egg were naturally insusceptible to this virus. We proceeded to inoculate grafts of human fetal membranes with the virus of mare abortion. The result was successful infection of human amniotic epithelium but failure to infect human chorion. This successful experiment demonstrated in the first place the susceptibility of human tissue to infection by the abortion virus, being the first demonstration of infection by it in unnatural host tissues;[3] and, in the second place, that, although the chick chorioallantois is itself not susceptible, that fact did not render the alien graft refractory.

It appeared that our failure to infect human fetal membranes with material from varicella and herpes zoster was not due to a deleterious influence exerted upon the grafts by the insusceptible chick embryo. The possibility remained that the human tissues were perhaps derived from persons who possessed an acquired immunity to these infections and factors responsible for such induced resistance had been carried over with the graft in sufficient amount to prevent infection. All of our experimental experience, however, has been opposed to such an inference. For example, we [4] have recently reported experiments concerned with grafting upon the chorioallantois skin from chickens immune to fowlpox. While a part of the immune fowl, this skin completely resists infection with fowlpox virus by direct inoculation, yet when it is removed and grafted upon the chorioallantois it becomes susceptible again and remains so when regrafted upon muscle of a normal chicken; but it rapidly regains a refractory state when regrafted upon the muscle of the immune chicken from which it was removed. This indicates that acquired resistance of epithelium is soon lost when it is removed from the immune animal and grafted on the chorioallantois. This conclusion is further substantiated by a few experiments with human skin grafts from persons immune to vaccinia. The epithelium from persons with an immunity acquired through vaccination can become susceptible to infection with vaccinia virus when grafted upon the chorioallantois of chick embryos.

We can conclude at the present time only that the human skin and fetal membranes which we have used possessed a degree of natural immunity that prevented infection of their epithelia with the viruses of varicella and herpes zoster, or that other limiting or inhibiting factors, concerning which we are at present ignorant, intervened.

Our experience thus far has shown that at least in the case of apparently complete refractoriness of epithelium to a particular virus there

is no susceptibility acquired from grafting upon a susceptible host. This is illustrated by our failure to infect human amniotic epithelium grafted upon the highly susceptible chorioallantois with the virus of fowlpox although the infected chick chorionic epithelium impinges upon the human amniotic cells (Fig. 4). There is reason to suppose, however, that human skin from a nonimmune individual and amniotic epithelium from human fetuses would be susceptible to the viruses of the human diseases, varicella and herpes zoster; and at present we are at a loss to explain our negative results.

An interesting fact, which concerns the relative susceptibility of amniotic as compared with chorionic epithelium to infection with the viruses of herpes simplex, vaccinia, variola and mare abortion, has appeared in our experiments with grafts from human fetal membranes, in that the amniotic epithelium is readily infectible while that of the chorion has remained, except the grafts from one placenta out of six, uninfected.

Little is known about these tissues in respect to infection and immunity, and the question of a difference in susceptibility to viruses will be the subject of further investigation. It is possible, of course, that the chorion, being immediately in contact with maternal blood or tissues, possesses a high degree of passive immunity conferred by the mother. Presumptively, however, this would hardly account for a difference in susceptibility to the virus of mare abortion; but as yet we know nothing about the nature and distribution of this virus except as an agent of equine infection.

Because the technic which we have used in making, inoculating and studying grafts of the human fetal membranes is of interest, it will be described in some detail.

Technic

Placentas are received directly from the obstetrical operating room in a sterile pan covered with a sterile towel. With sterile instruments an area of thin membrane is located in the neighborhood of the placenta. A sheet from 5 to 8 cm. square is cut out with scissors and spread upon a previously prepared sterile block of cork. Slabs of cork about 10 by 15 by 3 cm. are wrapped in gauze and paper and sterilized. Just before using the wrapping is removed and the gauze moistened with sterile saline solution. The gauze is then folded back, exposing the cork surface, and upon this the sheet of membranes is spread with the chorionic surface upward, if that membrane is to be used for grafting. Upon the exposed surface of the chorion lies a layer of decidua which is first stripped off with forceps. There remains a thin sheet of chorion and underlying amnion. These can be easily separated with the aid of forceps. The sheet of chorion is folded upon itself with the epithelial surface enveloped until used.

The chorionic membrane is then spread out on the cork with the epithelial surface uppermost and with a sharp scalpel it is cut into 1 cm. squares, as many as needed.

Eggs, incubated 9 to 11 days, should be prepared beforehand for opening by cutting a square window through the eggshell and coating the surface with melted paraffin.[5] They are then placed in an incubator with window up until ready for use.

The window is opened by cutting the shell membrane on three sides with a half spear-point needle and tearing off the dislodged shell from the fourth side. A puncture through the shell overlying the air-sac facilitates the dropping of the exposed membrane. A square of human membrane to be grafted is adjusted to the flat end of a small, sterile, searing iron of polished steel, with mesodermal surface exposed. The graft is then placed on the chorioallantois, dislodged from the searing iron and smoothed into place (Fig. 1). The window is closed in the usual way with a vaseline-paraffin ring upon which a coverglass is laid. The grafts are fixed at desired intervals by cutting out the chorioallantois to which they are attached, placing it on moist, absorbent paper and fixing in Zenker's fluid.

The procedure is the same when amniotic grafts are desired. The membranes are placed on the cork always with the surface that it is desired to graft upward. The two membranes are then separated, but that which is downward with the epithelium in contact with the cork is always discarded.

Bacterial contamination of grafts from placental membranes prepared in this way has been observed only very rarely.

Description of Grafts

The human membranal grafts readily become attached to the chick chorioallantois and survive throughout the period of incubation, under favorable circumstances. Both chorionic and amniotic membranes are very thin and avascular, and nourishment takes place apparently by plasmatic circulation, for there is no vascularization. Amniotic grafts appear in microscopical sections as a single layer of cuboidal or low columnar epithelial cells resting upon a hyaline basement membrane (Fig. 2). Beneath the basement membrane is a delicate layer of collagen containing rare nucleated cells. The chorionic layer is thicker. Its epithelial surface is composed of several thicknesses of its polygonal cells that rest upon a hyaline membrane, beneath which is avascular collagenous tissue containing few cells but relatively more than the amnion (Fig. 3).

Inoculation is usually performed about 24 to 48 hours after grafting. A paste of virus-containing tissue is applied directly to the presenting surface of the graft without scarification. The technic for making skin grafts has already been described.[1]

Results of Inoculations

Infection of Grafts of Human Tissue with Herpes Simplex Virus

In order to inoculate grafted human skin successfully it was necessary to snip the graft at several places with the points of sharp iris scissors and then to rub into these a fragment of infected tissue. In this way uniform infection of human skin grafts, inoculated 2 to 4 days after grafting, can be induced. Typical intranuclear inclusions occur in the infected epithelial cells, and sometimes vesiculation, pus-

tulation and ulceration ensue. The inflammatory reaction of the embryonic tissue is quite like that of the natural host.

Infection of amniotic grafts can be induced by direct application of fragments, or of a paste, of infected tissue to the exposed epithelial surface. Intranuclear inclusions occur in abundance; there is some hyperplasia; and infected multinucleated giant cells are at times conspicuous. With ensuing necrosis cellular reaction of inflammation and ulceration takes place (Figs. 7 and 8).

Although infection of amniotic grafts has followed inoculation with herpes virus quite consistently, that has not been the case following inoculation of chorionic grafts, either after direct application of virus, or after snipping the chorion. In fact, in only one series among numerous attempts with grafts from placentas has infection been demonstrated. In this case care was taken to strip off as much of the outer layer of decidua as possible before the grafts were cut (Fig. 9). Great care in this respect was taken with these grafts and because they were the last of the series, we cannot say with what frequency one might expect successful infection if this precaution is always observed. However, we believe that it is much more difficult to infect the chorion with the several viruses which we have used, than it is to infect the amnion.

In the successful series mentioned we also grafted thin sheets of stripped decidua. Sections of these showed much necrosis but no evidence of herpetic infection. The grafts did not survive well.

Infection of Grafts of Human Tissue with Vaccinia Virus

Inoculation of grafts of human skin with vaccinia virus following snipping gives rise to epithelial hyperplasia, Guarnieri bodies, pustule formation and ulceration. Infection of adult-skin grafts occurs quite regularly, even when skin from a person immunized by previous vaccination and retested for immunity is used. In these instances the grafts were allowed to remain on the chorioallantois at least 2 days before inoculation, and it is assumed that within this time any effective immune bodies were diluted by the plasmatic circulation so as to render them ineffective. Similar experiments have already been reported by us [4] in the case of skin grafts from chickens immune to fowlpox. The grafts became susceptible to fowlpox infection under these circumstances.

We have succeeded likewise in infecting human amniotic grafts, but the amniotic epithelium seems to be somewhat more resistant than cutaneous epithelium. Discrete, compact, basophilic, often multiple Guarnieri bodies are formed in the infected cells, and necrosis proceeds rather slowly (Fig. 10).

In only one instance, and that to a minimal extent, have we succeeded in infecting chorionic epithelium with vaccinia virus. This occurred in the same series of grafts in which herpetic infection was established, and in that series especial care was taken to strip off all of the outer coat of decidua.

Infection of Grafts of Human Tissue with Variola Virus

A strain of variola virus was isolated by Buddingh,[6] in 1938, from a mild outbreak of smallpox by inoculating the chorioallantois with pus from the cutaneous eruption. This strain has since been maintained continuously by membranal passage.

With material from one of the early passages, human-skin grafts were inoculated after snipping. Extensive infection resulted, with the formation of pustules. Cutaneous epithelium in the neighboring tissue contained an abundance of typical Guarnieri bodies. No characteristic nuclear changes or intranuclear inclusions were observed either in the human tissue or in chick embryonic cells (Figs. 5 and 6).

After maintenance in the embryonic membranes for more than 2 years, this strain of variola was used to infect human amniotic grafts. Typical Guarnieri bodies were formed, followed by necrosis, inflammation and ulceration. No typical nuclear changes were seen (Fig. 11).

In a single attempt with several grafts of chorion from one placenta no infection was induced. Here again the resistance of human chorionic epithelium seemed to be greater than that of the amnion.

Infection of Grafts of Human Amnion with the Virus of Mare Abortion

A certain type of abortion of mares was demonstrated by Dimock and Edwards [2] to be caused by a filterable virus. Abortion could be induced in mares by the injection of a filtrate of infected tissue from an aborted fetus. In cells of aborted fetal liver and lungs acidophilic nuclear inclusions were abundant and characteristic.[7]

With emulsified infected liver from aborted foals supplied by Dr. Dimock we have been able to infect epithelial cells of human amniotic grafts from four different placentas. Not all of a dozen grafts from a single placenta showed infection, but some of them from each case presented epithelial cells containing typical nuclear inclusions, associated with necrosis of others and ulceration with inflammatory exudation (Fig. 12). In no instance in grafts of chorion from the same human membranes was infection of chorionic cells observed.

Because embryonic cells of the developing egg are not demonstrably susceptible of infection with the virus of mare abortion, successful infection of human amniotic grafts has served to demonstrate not only

the susceptibility of human fetal tissue to this virus, but also that a susceptible tissue grafted upon the non-susceptible chorioallantois of the chick embryo does not thereby lose its susceptibility. Likewise an insusceptible tissue when grafted upon the chorioallantois does not become susceptible to a virus that readily infects the latter. This was shown by grafting human amnion onto the chorioallantois and inoculating it with fowlpox. Although the hyperplastic, inclusion-containing epithelium of the chick membrane impinged directly upon that of the amnion, no evidence of infection of the latter was discernible (Fig. 4).

Discussion

These experiments have demonstrated the possibility of studying infection of the component elements, amnion and chorion, of human fetal membranes by means of grafting them upon the chorioallantois of the developing chick embryo. Similar experiments have been successfully performed with grafts of human skin.

Little or nothing is known otherwise about the susceptibility and relative susceptibility of fetal membranes from the human embryo, although clinical observation has discovered intra-uterine infection of the human fetus by a number of viruses, notably those of variola, chickenpox and measles. The mechanism or pathogenesis of transmission of a virus from the mother to the fetus through the placental barrier is unknown; and when occasion arises this should be the subject of special study. A beginning in this direction is possible through the indirect method of grafting.

Although our experiments are not sufficient to warrant a definite conclusion, they at least suggest that chorionic epithelium is less susceptible to the viruses used than is that of the amnion.

Because it is well known that in the case, at least, of mothers immune to measles the placenta contains much antibody,[8] it is probable that more antibody is contained in the chorion and adnexed decidua than in the amnion; and the presence of antibody might to some extent increase the resistance of grafts from these tissues. An analysis of antibody content of the three tissues, amnion, chorion and decidua, so far as we know, has not been made.

If the amount of antibody is small, it will probably soon lose its effectiveness in grafts of immune tissue (skin) as has been demonstrated by Goodpasture and Anderson[4] for fowlpox, and as we have shown to be the case with skin from persons immune to vaccinia.

Our experiments with the virus of mare abortion have demonstrated the susceptibility of human amniotic epithelium to this virus; it is therefore a potential agent of human disease. We have likewise shown

that in respect to this virus, at least, the grafting of a susceptible tissue upon a non-susceptible chorioallantois does not render the graft insusceptible. Nor did an insusceptible tissue (human amnion) become susceptible to a virus (fowlpox) when grafted upon a susceptible chorioallantois.

Notwithstanding the fact that we have met with no success in our attempts to infect grafts of human skin, amnion and chorion with material from lesions of varicella and herpes zoster, we believe that further attempts should be made not only with material from these infections, but from other virus diseases.

A few preliminary experiments concerned with the application of theelin to human amniotic grafts gave some indication that the epithelium was better preserved and more active than otherwise. Further investigation of the use of hormones would be necessary, however, in order to estimate their effects upon prolonging the life and activity of grafted tissues and upon the susceptibility of their cells to viruses.

Summary

1. Human amnion and chorion have been successfully grafted upon the chorioallantois of the developing chick embryo.

2. Grafts of human skin, amnion and chorion were successfully infected with the viruses of herpes simplex, variola and vaccinia. The lesions induced were typical.

3. Human amniotic epithelium of grafts on the chorioallantois were infected with the virus of mare abortion.

4. It is concluded that a susceptible tissue grafted upon an insusceptible chorioallantois does not thereby lose its susceptibility; and an insusceptible tissue grafted upon a chorioallantois that is susceptible to a certain virus does not thereby become susceptible.

5. By means of grafts of fetal membranes a beginning may be made in a needed study of the pathogenesis of intra-uterine virus infections.

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DESCRIPTION OF PLATES

PLATE 89

FIG. 1. Human amnion (A) and chorion (B) grafted on the chorioallantois.

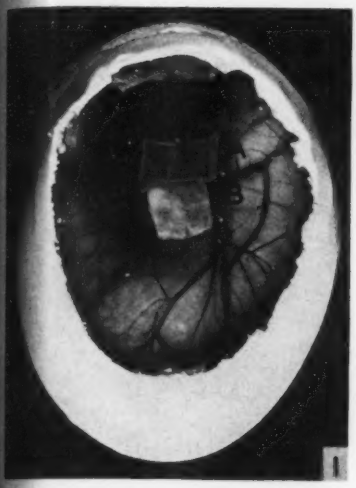
FIG. 2. Microscopical section of human amniotic graft (7 days). × 120.

FIG. 3. Microscopical section of human chorionic graft (8 days). × 120.

FIG. 4. Human amniotic graft (left) with its epithelium overlying chick chorionic epithelium infected with fowlpox. The amniotic epithelium is naturally resistant. × 325.

FIG. 5. Pustule in graft of human skin infected with the virus of smallpox. × 225.

FIG. 6. Margin of Figure 5 showing Guarnieri bodies in cutaneous epithelial cells. × 1400.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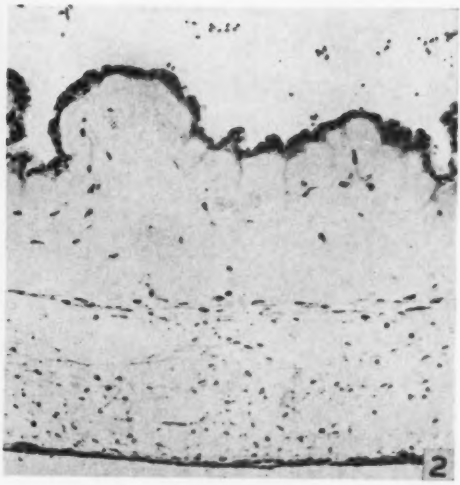
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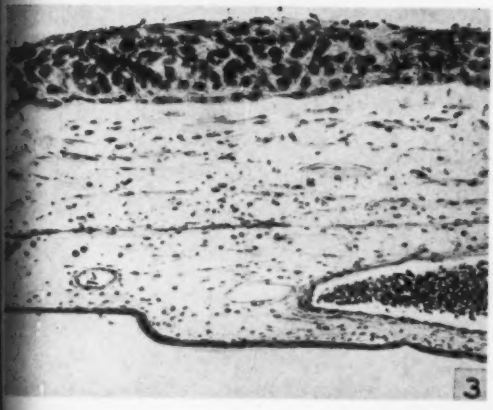
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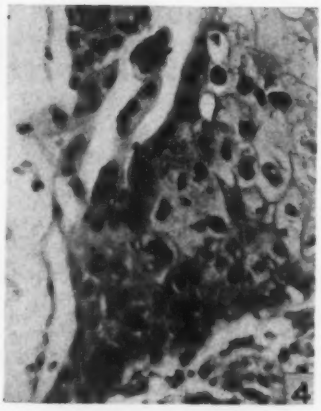
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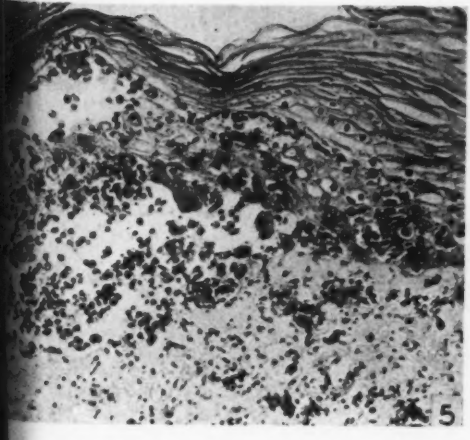
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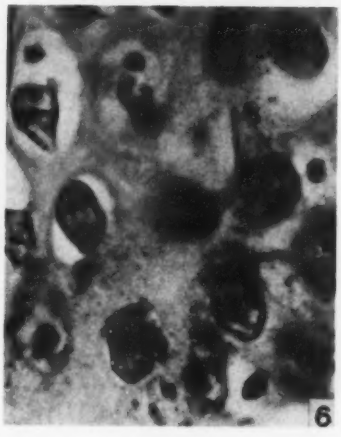
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PLATE 90

FIG. 7. Human amniotic epithelium of graft infected with the virus of herpes simplex. Intranuclear inclusions may be seen. × 1100.

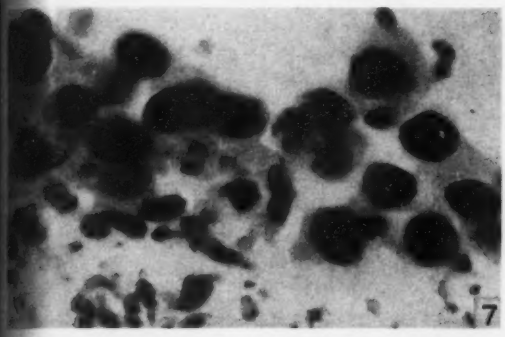
FIG. 8. Same as Figure 7, to show multinucleated giant cell of amniotic epithelium infected with the virus of herpes simplex. Intranuclear inclusions may be seen. × 1400.

FIG. 9. Human chorionic grafts infected with the virus of herpes simplex. Intranuclear inclusions are present. × 600.

FIG. 10. Human amniotic graft infected with vaccine virus, showing Guarnieri bodies in epithelial cells. × 1100.

FIG. 11. Human amniotic grafts infected with the virus of smallpox. Guarnieri bodies are present in epithelial cells. × 1600.

FIG. 12. Human amniotic graft infected with the virus of mare abortion. Intranuclear inclusions may be seen, some of which fill the nucleus. × 1200.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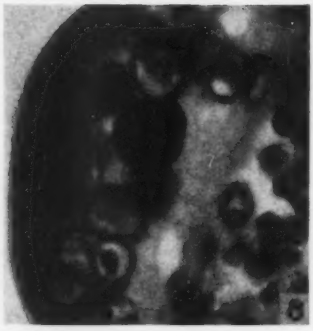
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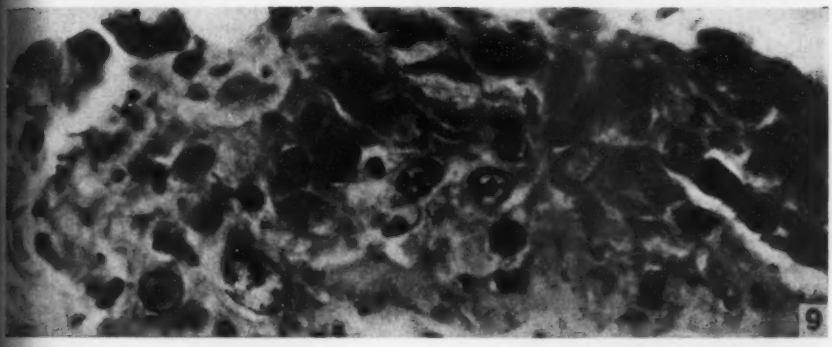
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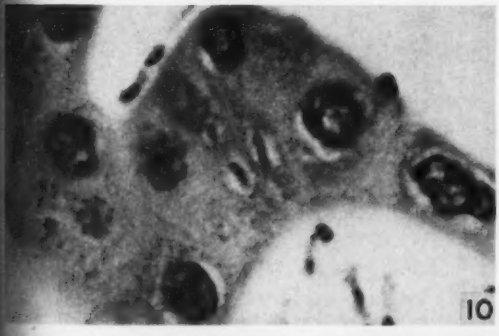
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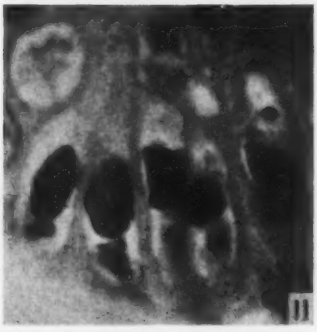
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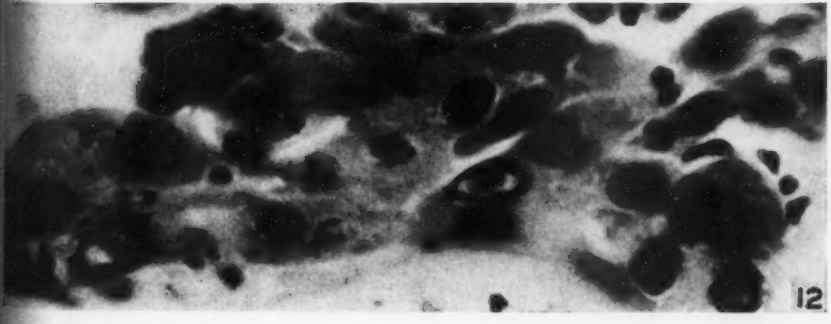
12

DUAL VIRUS INFECTION OF SINGLE CELLS *

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The hypothesis that activity of one infectious agent within a host may modify the course of disease instituted by another agent in the same host has long been proffered. Such an idea was hypothecated by Edward Jenner [1] after he had observed that the clinical course of vaccinal infection may vary with concurrent herpes simplex. The obligately intracellular parasitic nature of viruses makes the demonstration of multiple viral infections in single cells of some theoretical interest.

The following experiments establish the possibility of dual infection of single cells with different combinations of several viruses. Infections with the viruses of fowlpox, laryngotracheitis of fowls, vaccinia, herpes simplex and rabies are associated with the presence of specific intracellular inclusion-bodies.[2,3] Dual infection of single cells with certain combinations of these viruses have been determined by microscopical recognition of such specific inclusions. This concrete evidence of the activity of two viruses within one cell as presented by the occurrence of two different and characteristic kinds of inclusions in that cell establishes cytological evidence that single cells can become infected with more than one virus. These observations concur with those of Syverton and Berry [4,5] in which they describe coexistent infections of individual cells. These investigators induced specific inclusions of two viruses (herpes simplex and vaccinia) in single cells of rabbit's cornea.[6,7]

EXPERIMENTAL PROCEDURES

Technic

Chick embryos, 11 and 12 days old, served as experimental hosts. With the exception of one experiment in which nervous tissue was required, the chorioallantois was the organ in which dual viral infections were established. The source of each inoculum except that of rabies was a chorioallantoic membrane previously infected with a single virus. A suspension of embryonic chick brain infected with rabies virus † was used to establish rabic infections.[8]

Development of membranal lesions following inoculations with two viruses was watched through a coverglass over an opening in the egg

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† Supplied by Dr. James R. Dawson, Jr.

shell.[9] At varying intervals infected tissues were fixed in Zenker's fluid (5 per cent acetic acid). Histological sections were stained routinely with hematoxylin and eosin.

Fowlpox-Herpes Simplex Virus Infections

Chorioallantoic membranes of 11-day embryos were inoculated with fowlpox virus and incubated at 37° C. for 48 hours. The grossly evident fowlpox lesions were then inoculated with herpes simplex virus and were allowed to develop during 72 additional hours. During the last 3 days of incubation characteristics of fowlpox infection progressed and dominated the gross appearance of the lesions.

Microscopical examinations of sections presenting 5-day fowlpox lesions and concurrent 3-day herpetic infection showed intranuclear inclusions of herpes simplex and intracytoplasmic inclusions of fowlpox within single cells (Figs. 1 and 2). As is characteristic of infection with fowlpox virus, the ectodermal epithelium was markedly hyperplastic and hypertrophic. Almost all of the cells contained typical fowlpox inclusions. In some areas the intranuclear inclusions of herpes simplex occurred in the superficial layers of hyperplastic fowlpox-infected epithelium. At other foci the herpetic infection had spread through the whole epithelial layer of cells and had invaded the chorioallantoic mesoderm. It was determined that dual infection of single epithelial cells had occurred abundantly throughout the lesion.

It was more difficult to judge how the activity of one of these viruses in a cell had modified the influence of the other virus on the same cell. The appearance of the fowlpox inclusions and the effect of that virus on the cells appeared to be entirely typical of pure fowlpox infection. This is reasonable since the fowlpox infection, which develops more slowly and with less destruction, was given a 48-hour advantage over the herpetic infection. The intranuclear herpetic inclusions appeared larger and more basophilic than usual. The herpetic infection as a whole was less extensive and decidedly less destructive to cells than commonly. Fowlpox virus usually does not invade mesodermal cells, and herpetic infection of mesoderm apparently had not facilitated its invasion by fowlpox virus. In one case metastatic foci of herpetic infection were observed in the embryonic heart, as is characteristic of herpetic infection in chick embryos, without evidence of an accompanying metastasis of fowlpox.

Fowlpox-Laryngotracheitis Virus Infections

Chorioallantoic membranes were inoculated with fowlpox virus 24 hours before laryngotracheitis virus of fowls was superimposed, and lesions were fixed 72 hours later.

Microscopically the fowlpox infection appeared to have developed normally. The laryngotracheitis infection appeared well developed but was obviously imposed upon an abnormally hyperplastic epithelium. Intranuclear inclusions of laryngotracheitis and intracytoplasmic inclusions of fowlpox were readily determined to be present in single cells (Fig. 3). Laryngotracheitis virus normally stimulates the development of large multinucleated cells, each nucleus of which contains an inclusion. The characteristic feature of the combined infections was the occurrence of large syncytia whose nuclei contained typical laryngotracheitis inclusions and whose cytoplasm was filled with large masses of Bollinger bodies (Fig. 4).

If fowlpox and laryngotracheitis viruses are inoculated simultaneously, each virus invades epithelium and produces its characteristic inclusion. Dual infection of single cells is evident but the fowlpox lesion which normally develops more slowly is dominated by the more rapidly progressive lesion of laryngotracheitis.

Fowlpox-Vaccinia Virus Infections

Seventy-two-hour fowlpox lesions were inoculated with vaccinia virus and incubated for 48 and 72 hours longer. The epithelium was hyperplastic and practically every cell contained a fowlpox inclusion. Very few Guarnieri bodies were seen and it could not be determined that the cells were doubly infected. On the other hand, 24-hour fowlpox lesions inoculated with vaccinia virus and allowed to develop 48 hours longer showed exceptionally extensive vaccinal lesions with very few fowlpox inclusions. In this case, also, dual infection of single cells could not be determined with certainty. It appears that in the first series of experiments the vaccinia virus found an unfavorable environment for growth within cells well infected with fowlpox virus. In the second series one may reasonably speculate that fowlpox virus had parasitized a large number of epithelial cells and had stimulated a hyperplastic reaction; that vaccinia virus, gaining entrance to these hyperactive cells before the slowly forming fowlpox inclusions were developed, found a favorable medium for growth and that the rapid development of Guarnieri bodies prevented almost entirely the appearance of fowlpox inclusions. Since inclusions of both these viruses are cytoplasmic, a dual infection of single cells is more difficult to recognize.

Herpes Simplex-Vaccinia Virus Infections

Membranes inoculated with mixed suspensions of herpes simplex virus and vaccinia virus were fixed after 48 hours of incubation. There was considerable necrosis and ulceration of epithelium. Microscopically, infection with each virus was evidenced by the occurrence of

characteristic inclusions. Areas in the epithelium where the infection appeared to be only vaccinal merged into other foci which were purely herpetic. It was at these margins where dual infection of single cells could be most clearly demonstrated (Fig. 5). Such doubly infected cells occurred but rarely. Although the development of an herpetic and a vaccinal inclusion within one cell did occur, it was an unusual rather than a characteristic feature of these lesions. Each virus metastasized from these complicated membranal lesions to the liver of the embryos. Vaccinal foci and herpetic foci in the liver seemed to occur independently of each other.

Rabies-Herpes Simplex Virus Infections

Twelve-day-old embryos were inoculated intracerebrally with 0.03 cc. of 1:20 suspension of chick embryo brain infected with rabies virus. After 72 hours of incubation the same embryos were given a similar inoculation of chorioallantoic tissue infected with herpes simplex virus. After another 72 hours of incubation the embryos were sacrificed for histological study. Grossly the embryos showed a marked hydrocephalus, which Dawson [10] has reported as being characteristic of rabic infection in chick embryos.

Microscopically there was extensive destruction of brain substance with somewhat less hemorrhage than is found in herpetic encephalitis. Sections stained with eosin, fuchsin and methylene blue showed intranuclear herpetic inclusions in a great many cells that also contained typical Negri bodies in their cytoplasm (Figs. 6 and 7).

Other Attempted Dual Infections

On the assumptions that the growth of a virus within a cell depends upon the furnishing by that cell of a favorable nutritional and physiological environment for the activity of the virus, and that the growth of a virus in a cell alters the normal physiological activity of the host cell, the possibility that an already parasitized cell might be susceptible to infection by a virus to which a normal cell is resistant was considered. In these experiments, fowlpox virus was used as the primary infectious agent because it is slow to cause necrosis of individual cells.

The viruses of varicella, herpes zoster, of abortion of mares and the agent of an inclusion pneumonia in human beings have not as yet been shown to be cultivable on embryonic chick tissues. Material that might have contained one of each of these viruses was inoculated onto chorioallantoic membranes infected with fowlpox virus with the idea that a fowlpox-infected cell might offer an altered and favorable environment for the growth of one of these other viruses.

Microscopical sections showed no evidence of the growth of any of these agents except fowlpox virus itself.

Discussion

By using viruses that form recognizable intracellular inclusions, we have been able to demonstrate cytological evidence that individual cells may be invaded by, and become hosts to, two different viruses. These observations have been concerned chiefly with the parasitized cells themselves and not with the influence that one infection may have on another and concurrent infectious process in the same host. The combinations of viruses; *viz.*, fowlpox with herpes simplex, with laryngotracheitis and with vaccinia, and herpes simplex with vaccinia and with rabies, were chosen so that the occurrence of intranuclear and intracytoplasmic inclusions in one cell could be assuredly interpreted as representing dual infection of that cell.

The viruses of herpes simplex and laryngotracheitis appear to grow well within fowlpox-infected cells and these secondarily imposed infections are progressive. The same is true of herpetic infection in rabies-infected cells. Fowlpox and rabies viruses are characterized by their slow destruction of cells. On the other hand, two viruses like herpes simplex and vaccinia that very rapidly change the physiological activity of cells are by the method used only rarely found to infect one and the same cell. In no instance has it appeared that the activity of one virus within a cell rendered it more readily susceptible to invasion by another virus.

Findlay and MacCallum [11] presented as "a possible explanation of the interference shown by the neurotropic strain of yellow fever virus with the pathogenic action of the pantropic strains of yellow fever and Rift Valley fever" the idea that "when certain cells are already occupied by actively multiplying virus particles they cannot be invaded by certain other virus particles." The same interference phenomenon has been observed in experiments with certain related plant viruses.[11] Such an interference phenomenon is but rarely encountered, and investigators agree that only strains of the same virus or closely related viruses are likely to exhibit it. The mechanism of the interference is obscure.

The viruses used in this study were not related, nor does infection with any one of them protect a natural or experimental host against invasion by any other of them. These experiments show that certain different viruses may invade and multiply within single cells. It is only to this extent that the present discussion has any bearing on an interpretation of the interference phenomenon.

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DESCRIPTION OF PLATE

PLATE 91

FIG. 1. An ectodermal epithelial cell of chick chorioallantois showing an intranuclear inclusion of herpes simplex and a cytoplasmic inclusion of fowlpox. Five-day fowlpox lesion with concurrent 3-day herpes simplex. × 3000.

FIG. 2. A single epithelial cell showing a diffuse, granular, herpetic inclusion in the nucleus and a characteristically lobulated fowlpox inclusion in the cytoplasm at the left of the nucleus. Five-day fowlpox lesion with concurrent 3-day herpes simplex. × 3000.

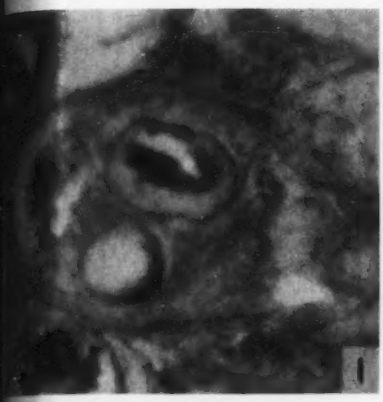
FIG. 3. A single epithelial cell showing intranuclear inclusion of laryngotracheitis and cytoplasmic inclusion of fowlpox. Four-day fowlpox lesion with concurrent 3-day laryngotracheitis. × 3000.

FIG. 4. A multinucleated cell showing five nuclei containing inclusions of laryngotracheitis, with numerous fowlpox inclusions in its cytoplasm. Four-day fowlpox lesion with concurrent 3-day laryngotracheitis. × 2200.

FIG. 5. Epithelial cells showing two intranuclear herpetic inclusions of the diffuse type and two Guarnieri bodies of vaccinia. One cell infected with herpetic virus has in its cytoplasm a large vaccinal inclusion within a vacuole. Forty-eight-hour infection. × 3000.

FIG. 6. A single neuron in the brain of an embryonic chick showing a diffuse, intranuclear, herpetic inclusion and a small Negri body in its cytoplasm. Six-day rabic infection with concurrent 3-day herpetic encephalitis. × 2200.

FIG. 7. A single ganglionic nerve cell showing an intranuclear herpetic inclusion and a large Negri body in its cytoplasm. Six-day rabic infection with a concurrent 3-day herpetic encephalitis. × 2200.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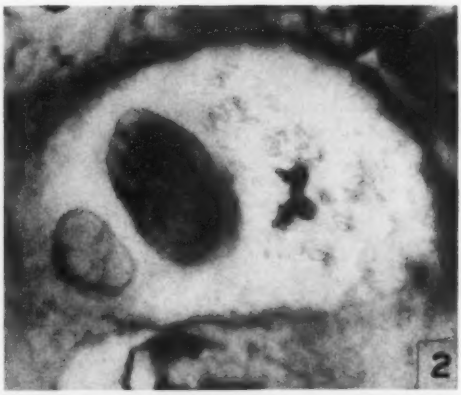
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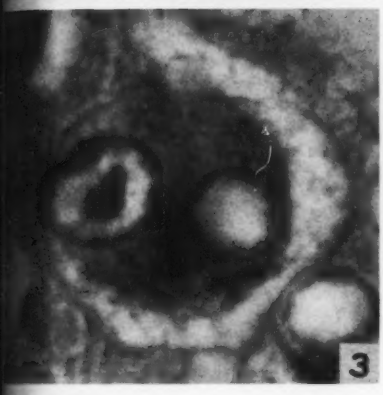
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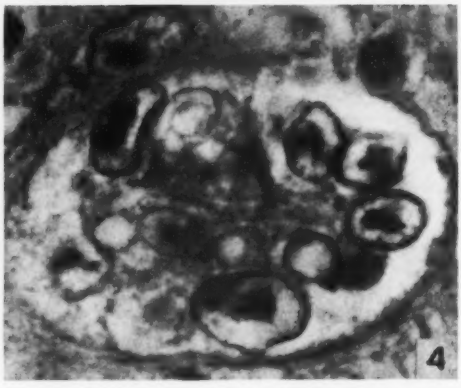
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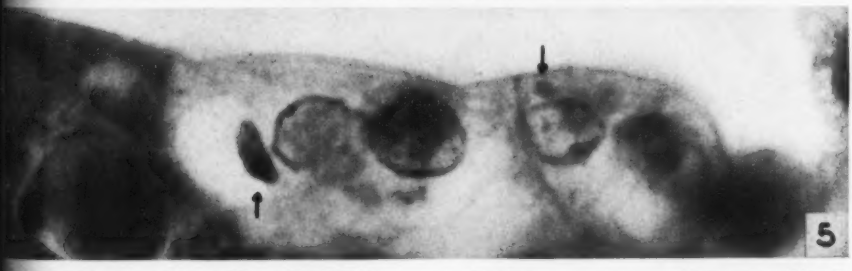
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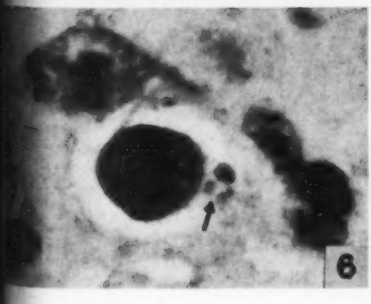
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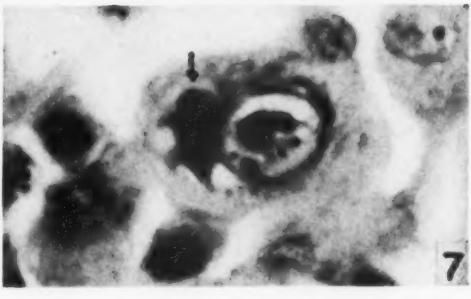
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DEMONSTRATION OF THE FORMATION OF RETICULIN BY SCHWANNIAN TUMOR CELLS IN VITRO *

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In a recent communication we reported the *in vitro* cultural characteristics of Schwannian cells grown from normal nerves and from neurilemomas and demonstrated to our own satisfaction that neurilemomas are tumors composed of Schwannian cells. We inferred that the collagen and reticulin fibers in neurilemomas were formed by Schwannian cells, although at that time this had not been demonstrated *in vitro*.

More recently, by variations in technical methods, we have succeeded in demonstrating the formation of reticulin by Schwannian cells from a mediastinal tumor grown *in vitro*. This confirms the inference previously expressed and offers an entirely satisfactory explanation for the presence of connective tissue fibers in tumors of purely Schwannian origin.

The patient from whom the material was obtained was a female, 49 years old, who had had first interscapular pain and later paraplegia of the lower extremities for 4 years. A dumb-bell-shaped tumor was found which compressed the cord in the region of the fourth thoracic vertebra and extended through the intervertebral foramen to the right posterior mediastinum. The portion compressing the cord was first removed and 21 months later the mediastinal prolongation was excised. Two years after the first operation, and 3 months after the second, the patient was well and free from symptoms.

The tumor was encapsulated and the second portion, removed from the mediastinum, measured 7 by 5 cm. It was solid, with a firm, fibrous peripheral zone and a large softer center which was tinted yellow because of its large lipoid content. This tumor varied in its histological appearance in different parts. In the central area it had the characteristic appearance of a neurilemoma divided into A and B tissues with an unusually large number of phagocytic foam cells filled with lipoid. The Schwannian nuclei tended to be aligned in palisades but nowhere was there a definite organoid arrangement. Here there were extremely few reticulin fibers. In the peripheral zone there were few B areas and almost no lipoid. In this region the great bulk of the tissue was composed of Schwannian cells closely packed in

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masses and accompanied by many collagen and reticulin fibers. Some of these areas closely approached the appearance of neurofibroma. Chloral hydrate fixation and Cajal impregnation failed to show any neurofibrils. We were of the opinion that the tumor should be classified as a neurilemoma but it demonstrated what we have observed before—that neurofibroma and neurilemoma are in fact only different growth manifestations stemming from a single basic origin, namely, the Schwannian syncytium. Blood vessels consisting of endothelial lining and a rather thick collagenous sheath were scattered irregularly throughout the tumor.

The tissue used for explantation came from the periphery and included a larger proportion of the solid fibrous zone and a smaller amount of the lipoid-containing central area of characteristic neurilemomatous aspect.

Methods

The Maximow double-coverslip method of handling a hanging, or rather, a lying drop was used exclusively in the tissue cultures. These were explanted in a medium composed of 2/10 chicken plasma, 5/10 human placental serum, 1/10 chicken embryo extract and 2/10 serum ultra-filtrate, prepared by Dr. H. S. Simms from beef blood. The use of this medium reduced to a considerable extent the liquefaction of the clot which usually accompanies the growth of these tumors *in vitro;* so that it was generally possible to keep the cultures in their original situation on the coverslip without transferal throughout the period of cultivation. At intervals of 2 to 4 days they were washed in a buffered saline solution (Simms and Sanders) and the liquid components of the medium were subsequently renewed. Occasionally they were patched with fresh chicken plasma. For the washing it was found convenient to set the cultures vertically in small, covered Coplin jars designed to hold coverslips.*

At intervals during the 57-day period of cultivation, cultures were fixed in Zenker's or Helly's fluids and stained with Delafield's or phosphotungstic acid hematoxylin or with fuchsin-ponceau and aniline blue. Zenker's fluid was found to be the most favorable fixative for preceding silver impregnation for reticulin. For this the Bielschowsky method was used, as adapted for tissue cultures by McKinney from Foot and Mènard's modification. In all these procedures, coverslip Coplin jars were employed and the cultures handled in an upright position, which greatly facilitates drainage. The silver impregnations were counterstained lightly with toluidine blue, in 0.04 per cent aqueous solution.

* These jars, which are exceedingly useful in the various operations of tissue culture, are purveyed by the Arthur H. Thomas Co. of Philadelphia.

Growth Characteristics in Vitro

In vitro this mediastinal neurilemoma produced a characteristic Schwannian outgrowth of the same general type as that exhibited by other nerve sheath tumors which we have studied, and by normal adult nerves (Fig. 3). As already stated, the explanted tissue was composed chiefly of A-type tissue; this constitution was confirmed in the tissue cultures, which gave rise to almost no B cells.

Within 3 days after explantation the A type of Schwann cell began to grow. This cell at first appeared in single, filamentous formation resembling some forms of neuroglia, but was often multinucleate. Later it thickened, forming a ribbon or a bundle of parallel, anastomosing filaments (Fig. 2), which still later, after a month or so of cultivation, might develop into a bundle or tuft of ribbons (Fig. 1).

Characteristic of these bundles were their lateral anastomoses. In the tissue cultures there was an almost complete absence of the compression which usually obscures the relationships between these cells *in vivo*, and this permitted illuminating observations of their growth characteristics. By continuous observation, fasciculated bundles could be seen to form in essentially the same manner as postulated by Masson from the study of a tumor nodule distended by edema. Figure 2 might serve as the source of one of his diagrams. Palisading of the nuclei often occurs *in vitro*, but usually does not last over a long period, possibly because of the freedom of movement allowed the cells by their roomy environment.

After about 10 days *in vitro* some of the cultures produced, in addition to the A cells, a semimembranous outgrowth composed of broad, flat cells resembling endothelium which were usually to be found on the surface of the clot, as contrasted with the Schwannian cells which characteristically push their way through the clot. When treated with silver nitrate this semimembranous outgrowth was shown to form a mosaic of cells with blackened cement borders—characteristic of endothelium but not of Schwannian cells. It was therefore supposed that such cells were derived from the blood vessels of the tumor.

A 5-year study of human and animal, fetal and adult, normal and abnormal nerve sheath tissue *in vitro* has convinced us that Schwannian cells can be distinguished positively from fibroblasts in this medium, on the basis of growth characteristics and pattern, and on general morphology and physiology. We have no hesitation, therefore, in stating that some cultures, and many areas in all the cultures, were entirely free from cells of fibroblastic origin. Only such areas will be considered in the following discussion of fiber formation by Schwannian cells *in vitro*.

Reticulin Formation by Schwannian Cells

Reticulin formation in these cultures was slow, sparse and sporadic. The reticulin appeared first as a row of fine rods and granules which later coalesced to form long, single fibers running parallel to the long axis of the Schwann cell. The fiber always originated in close relationship to the cell, often appearing to lie in contact with the long-drawn-out cytoplasmic portion and making a curved detour around the lateral surface of the nucleus. Growth and migration of cell or bundle sometimes left these fibers behind without connection with any cell, but they did not so originate. They always appeared to develop singly, not as the mat of fibers described by McKinney in the mammalian lymph node, or as bundles of fibers described by Stearns in rabbit connective tissue.

In general, the reticulin fibers shown by silver impregnation were not in contact with fibers in the explant, but occupied a zone adjacent to the explant. Fibers which stained with aniline blue were always in contact with old fibers in a zone of dense outgrowth, so that it was difficult to be certain where the new growth of fibers began.

The first argyrophil fibers were observed in a 13-day culture. They were present in some older cultures and not in others. There was no orderly progress in fiber formation among the cultures as a whole. The speed and intensity of the process varied from culture to culture, as it appears to do in various areas of a tumor. By comparison, cultures of the stroma of an adenomatous human parathyroid gland, cultivated in the same medium under similar conditions, laid down reticulin fibers very much more densely, regularly and rapidly. Such fibers appeared first as criss-cross bundles in contact with the explant (Fig. 6).

In the neurilemoma cultures, reticulin was rarely seen in connection with macrophages or structures believed to be derived from blood vessels present in the explants. Great care was taken to exclude all such areas from consideration in the material used for the foregoing description of reticulin formation around Schwannian cells, and only cells and bundles which could be positively identified as Schwannian were included. Figures 4 and 5, showing reticulin, represent cell formations essentially similar to those of Figures 2 and 1, in which the cell structure and arrangement are brought out with hematoxylin staining.

Summary

Reticulin fibers have been shown to arise *de novo* in connection with Schwannian cells growing out from a mediastinal neurilemoma cultivated *in vitro*. The areas under consideration were devoid of cells of

fibroblastic derivation. The process of fiber production by Schwannian cells differs in certain respects from fiber production by cells of mesoblastic origin.

NOTE: We are greatly indebted to Irene Bokoff for technical assistance in the handling of the tissue cultures, and to Walter I. O'Neill for the photography.

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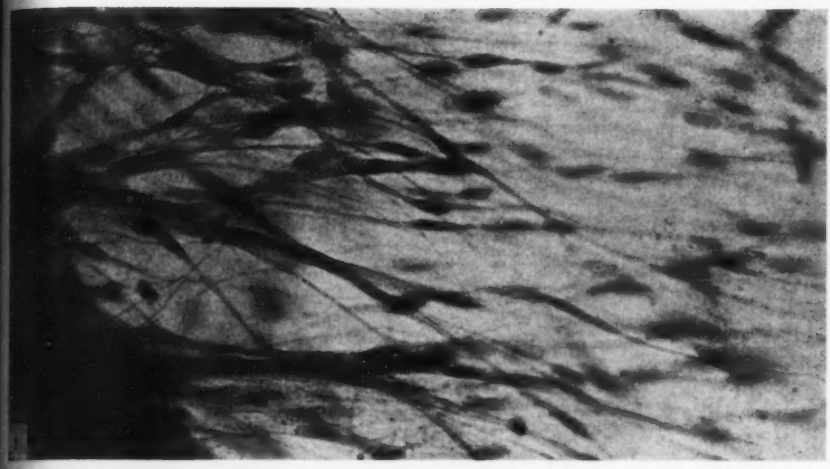
DESCRIPTION OF PLATES

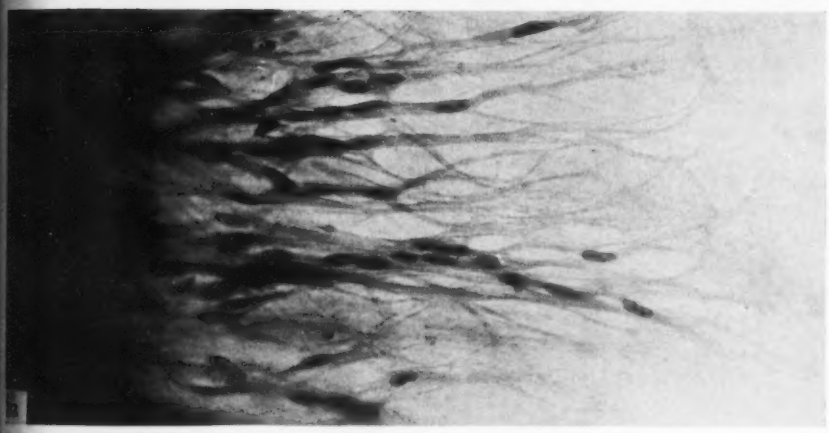
PLATE 92

FIG. 1. Neurilemoma from mediastinum (no. 77393) after 24 days *in vitro*, showing Masson's fasciculated bundles of Schwann cells. Zenker's fluid, Delafield's hematoxylin stain.

FIG. 2. Neurilemoma from mediastinum, after 15 days *in vitro*, showing "aneuritic bundles" of Schwann cells, expanded in one plane. Helly's fluid, Delafield's hematoxylin stain.

FIG. 3. Schwannian outgrowth from small medullated fibers in normal human adult celiac nerve, after 24 days *in vitro*. Carnoy's fluid, Delafield's hematoxylin stain.





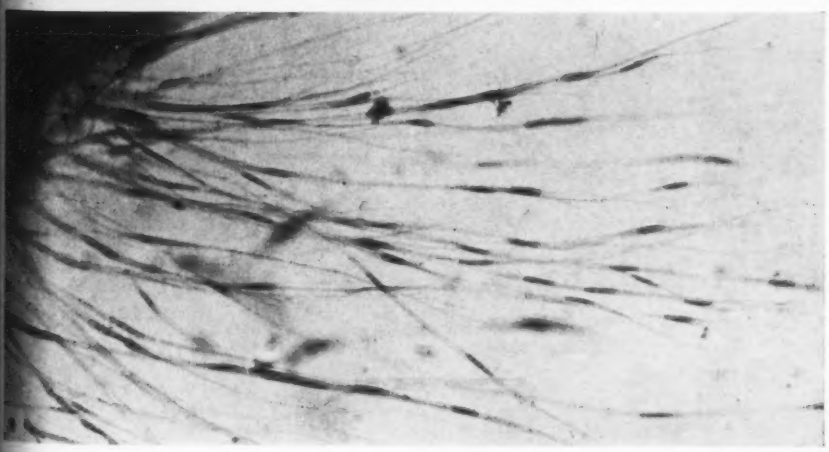


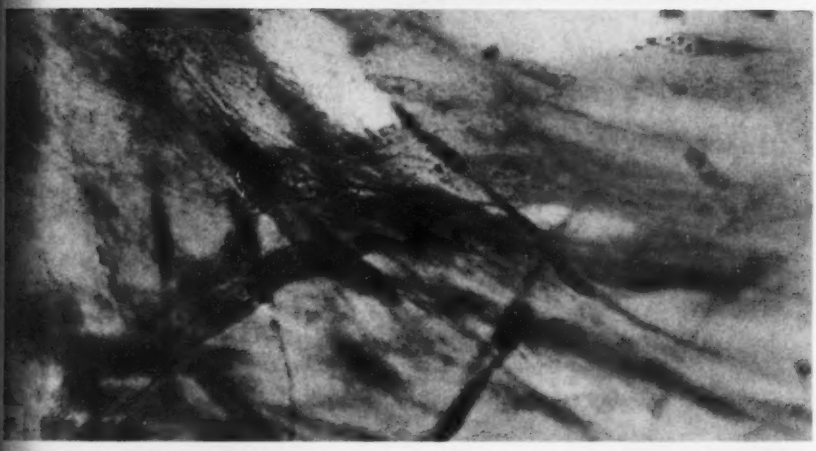
PLATE 93

(All three photomicrographs were taken at the same magnification, with high dry objective lens.)

FIG. 4. Neurilemoma from mediastinum (no. 77393) after 24 days *in vitro*, showing longitudinal reticulin fibers formed between Schwann cells. Zenker's fluid, Foot's silver impregnation.

FIG. 5. Similar to Figure 4, showing longitudinal fibers in a bundle of Schwann cells like those of Figure 1.

FIG. 6. Parathyroid adenoma (no. 76598) after 20 days *in vitro*, showing matted reticulin fibers formed among fibroblasts from the stroma of the tumor. Zenker's fluid, Foot's silver impregnation.



AN ANATOMICAL STUDY OF THE CLOSURE OF THE DUCTUS ARTERIOSUS *

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The successful surgical ligation of a patent ductus arteriosus [1] has aroused new interest in this structure. This report concerns an anatomical study of a series of ductus arteriosi in an attempt to determine the mechanism of closure.

The method of closure of the ductus arteriosus has long been a disputed point. The approach to this problem resolves itself into the factors concerned with the immediate physiological closure occurring shortly after birth and into the subsequent anatomical obliteration of the lumen.

The early concept that thrombosis was the immediate cause of closure has been discarded. Thrombi have been demonstrated rarely in the ductus arteriosi of infants [2,3] but are considered abnormal. Rauchfuss [4] found the ductus arteriosus thrombosed only four times in 1,400 infants. The ingenious theory of Strassmann [5] postulated that immediate closure of the ductus arteriosus is effected by means of a valvelike intimal fold situated at its aortic orifice. After birth the increased blood pressure in the aorta closes the valve, preventing reflux into the pulmonary artery. It has been well established that the ductus arteriosus enters the aorta at an acute angle and that the entrance into the pulmonary artery is less acute. Roeder [6] found that in newborn infants the angle of entrance of the ductus arteriosus into the aorta is always an acute angle of about 33 degrees. This has been confirmed by Gräper.[7] Strassmann, and later Fromberg,[8] carried out a number of injection experiments in infants which purported to show that the ductus arteriosus could not be injected from the aortic side with greatly elevated pressure, although the injection from the pulmonary side allowed easy passage of fluid through the ductus arteriosus into the aorta. This work was regarded as proof of the effectiveness of the Strassmann valve. Fromberg pointed out the similarity of the entrance of the ductus arteriosus into the aorta with the entrance of the ureters into the bladder. The results of injection technic and the effectiveness of the Strassmann valve have been disputed by Gräper, Linzenmeier [9] and others. Thus Gräper believed the elevated intimal fold at the angulated junction of the ductus arteriosus with the aorta too small to be effective.

* Received for publication, August 21, 1941.

Schanz [10] believed immediate closure of the ductus arteriosus resulted from stretching and knuckling of this structure produced by a change in position of the thoracic organs effective with the onset of respiration. Linzenmeier [9] made careful comparative anatomical studies of stillborn fetuses and infants who had died after breathing and believed he could show a definite change in position of the heart and great vessels after respiration with resulting knuckling of the ductus arteriosus. In addition, he injected the ductus arteriosus of stillborn infants, then inflated the lungs and obtained moulds which demonstrated a knuckling of the ductus arteriosus following insufflation. This work was criticized by Gräper,[7] who made similar studies and concluded that while there was a knuckling of this structure with full inspiration, the knuckling was much less pronounced with expiration and therefore could not be the responsible factor in immediate closure. A mechanism similar to that postulated by Schanz was that of Melka,[11] who presumed that at birth the pulmonary artery and aorta become distended with blood and compress the ductus arteriosus, producing immediate closure.

On the basis of the histological structure of the wall of the ductus arteriosus, effective closure by active muscular contraction has been a popular concept, supported by Gräper,[7] von Hayek,[12] Swensson [13] and many others. Thus von Hayek was impressed by the similarity in structure of the muscle fibers of the umbilical artery and of the ductus arteriosus. Gräper was of the opinion that the same stimulus which leads to contracture of the umbilical artery also leads to contracture of the ductus arteriosus. Recently new impetus has been given to this theory by the work of Barclay, Barcroft, Barron and Franklin.[14,15] These workers used lamb fetuses delivered by cesarean section, leaving the maternal circulation intact and preventing respiration by holding a rubber bag containing amniotic fluid over the snouts of the fetuses. Radio-opaque dye was injected into the external jugular veins or the umbilical arteries of the fetuses and the outlines of the heart and great vessels were visualized and recorded with motion pictures. It was found that the ductus arteriosus of these fetuses became closed soon after birth (in one case, in 5 minutes) and that this closure occurred even in the absence of respiration and without interruption of the maternal circulation. In several instances they were able to force the dye into the femoral arteries of moribund fetuses in which the maternal circulation had been interrupted. The dye extended in a retrograde fashion into the aorta, ductus arteriosus and coronary arteries. From their studies they concluded that the pulmonary end of the ductus arteriosus is the first portion to close.

Confusion exists as to the histological structure of the ductus arteriosus in the newborn infant and in the subsequent anatomical changes leading to closure of the lumen. Rokitansky [16] thought there was no difference in the histological structure between the ductus arteriosus and the aorta. Subsequent investigators (Gräper,[7] Linzenmeier,[9] Melka,[11] von Hayek,[12] Swensson [13] and many others) have shown this to be incorrect. There is general agreement that the ductus arteriosus of the newborn is looser in structure than the great vessels, that it has a well defined internal elastic lamina, has less elastic tissue than the great vessels, and that it has no external elastic lamina. There are conflicting views as well. Thus the direction of the muscle fibers in the media of the ductus arteriosus was described by von Hayek as running in spiral lines. Gräper described an inner longitudinal layer of muscle fibers and an outer circular layer with interspersed longitudinal bundles. Melka found an inner and outer longitudinal layer and a central circular layer.

Swensson [13] made a careful histological study of a series of ductus arteriosi taken from human fetuses ranging from 3.5 to 47 cm. in length. Even in a 4.5 cm. fetus he observed that the ductus arteriosus had less elastic tissue and was looser in structure than the aorta and pulmonary arteries. In a 10 cm. fetus there was a well defined internal elastic lamina. In this fetus there was abundant collagen in the media of the pulmonary artery and aorta, yet there were only a few collagen strands in the media of the ductus arteriosus and these lay in the outer portion of the media. In a 23 cm. fetus he noted intimal pillows, *i.e.*, eccentrically thickened intimal mounds composed of smooth muscle and elastic tissue. Beneath these mounds the internal elastic lamina was frequently fragmented and split. The pillows persisted in progressively older fetuses. From the 23 cm. fetus to the full-term infant there was no significant change in the structure of the ductus arteriosus. Gräper [7] found that the ductus arteriosus of the newborn infant possesses thick intimal mounds. He pointed out the presence of holes in the loose inner portion of the media. These holes are situated most frequently near the bases of the intimal mounds and often contain precipitated plasma and cellular elements of the blood. Gräper showed that with progressing age there are changes in the structure of the ductus arteriosus. The intimal pillows become thicker, with resulting narrowing of the lumen. The media becomes thicker and more compact and the holes in the media vanish. As early as 8 days after birth there is evidence of ischemic degeneration in the inner portion of the media with subsequent progression to include the intima and its mounds. There is a relative increase in elastic tissue due to degeneration of other structures. The lumen is visible for a period

of 1½ years but is occluded at each end. Throughout life the characteristics of a vessel are maintained. Melka [11] explained the closure as follows: Obliteration is a proliferative process, which he observed beginning as early as in a 5.5 cm. fetus. The internal elastic membrane proliferates in an asymmetrical fashion, forming mounds which project into the lumen. Gradually collagen grows from the mounds out into the lumen, effecting complete closure. Weizmann [17] stated that in old age, calcification, cartilage and fat frequently appear in the course of the ductus arteriosus. Benninghoff [18] observed that in the proliferating process, elastic fibers and connective tissue participate. In the scar of the ductus arteriosus in old age there is frequently calcium deposition. A stellate-shaped lumen most frequently remains in the central portion. Finally the structure is converted into a connective tissue strand in which elastic fibers and smooth muscle persist.

The time required for complete closure of the ductus arteriosus is variable. Mönckeberg [19] stated that any ductus arteriosus which remains open longer than 3 months should be regarded as patent, but added that occasional cases occur in which closure is effected in the second and third year. Christie,[20] in a series of 558 autopsied infants, found that at 2 weeks, 64 per cent of the ductus arteriosi were still patent; at 6 weeks, 20 per cent; at 12 weeks, only 5 per cent, and by the end of a year, only 1.2 per cent were still patent. Vierordt [21] considered the ductus arteriosus obliterated at the end of 20 days. Théremin [22] found that the ductus arteriosus was open in 23 per cent of 144 infants varying from 1½ to 4 months of age. Théremin observed that the obliterative process begins in the middle of the ductus arteriosus, progresses next to the pulmonic end and finally to the aortic end. According to Rokitansky,[16] the pulmonic end narrows first.

Gerhardt [23] classified patent ductus arteriosus in adults into four types: (1) cylindrical type, (2) funnel-shaped type with the dilated portion at the aortic orifice, (3) window type with little or no separation between the aortic and pulmonary orifices, and (4) an aneurysmal dilatation of any portion. In addition there is a fifth type in which there is incomplete patency with closure occurring only at the pulmonic end. This type has been described by Hebb,[24] Mönckeberg [19] and Altschule.[25]

Material and Methods

Our material is composed of a series of 71 ductus arteriosi representing a range from 28 cm. fetuses to adults 80 years of age. The specimens were fixed in Zenker's solution. The loose connective tissue was then removed from the vessel wall, measurements made and sections

taken. The usual procedure was to take transverse sections at the aortic and pulmonic ends of the structure and also a transverse section of the central portion. Frequently, however, longitudinal sections of the pulmonic and aortic ends of the ductus arteriosus and transverse sections of the central portion were taken. The longitudinal sections included portions of the adjacent pulmonary artery and aorta. Sections were stained with phloxine and methylene blue and with Verhoeff's elastic tissue stain, using the van Gieson counterstain.

Gross Findings

All except 5 in this series of 71 ductus arteriosi were cylindrical in type. The acute angulation at the junction with the aorta was a constant occurrence. The ductus arteriosus varied from 0.5 to 1.3 cm. in length and from 0.1 to 0.8 cm. in diameter, after fixation. The average measurements in this series were 0.8 cm. in length and 0.3 cm. in width. Anatomical closure usually occurred by the end of the third week of life. An occasional vessel was found to be patent as late as the end of the second year. Three ductus arteriosi of the window type were observed in which the aortic and pulmonic orifices were closely approximated without any demonstrable intervening structure which could be identified as a vessel. All specimens of the window type were widely patent. These were obtained from infants of 3 days, 4 days and 3½ months of age. In addition there were two adults with a ductus arteriosus of the cylindrical type closed only at the pulmonic end.

Histological Findings

The ductus arteriosus presents a histological appearance similar to that of any systemic artery of equal size, with an intima, well defined internal lamina, media and adventitia. By intima is designated the portion of vessel wall lying internal to the internal elastic lamina. The intima is peculiar in that there are mounds which project into the lumen. These mounds, which contain fine elastic fibers, smooth muscle and later collagen, are an integral part of the mechanism of closure. They vary in number, width and length in a given cross section and in different portions of the same vessel. The media, in contrast to that of the aorta and pulmonary artery, is loose in structure and contains fine, wavy elastic fibers rather than coarse longitudinal bundles. No external elastic lamina is present in the ductus arteriosus. The adventitia, poorly delimited from the outer portion of the media, contains collagen, elastic fibers, smooth muscle and small blood vessels.

The peculiar elastic structure of the ductus arteriosus in contrast to that of the aorta and pulmonary artery is effectively shown in longi-

tudinal sections of this vessel and the adjacent great vessels. The elastic fibers of the aorta and pulmonary artery condense into a coarse elastic band at the orifice of the ductus arteriosus. The major portion of the elastic fibers traverses obliquely to the adventitia at each end of the ductus arteriosus and terminates. A small portion forms the internal elastic lamina of the ductus arteriosus and the loose, fine meshwork of elastic fibers of the media.

Two 28 cm. fetuses were sources of the earliest specimens in this series. In these the lumina were widely patent. The thick internal elastic lamina was subendothelial except in several areas where low mounds projected slightly into the lumen. These mounds, which were composed of smooth muscle and moderate numbers of fine, wavy elastic fibers, appeared to arise from a splitting and reduplication of the underlying internal elastic lamina. The media was loose, thin, and consisted of smooth muscle and fine, wavy elastic fibers. Just beneath the internal elastic lamina the muscle fibers of the media had an oblique or radial arrangement which was most marked beneath the intimal mounds. Longitudinal muscle fibers flanked the radial fibers. The muscle fibers in the outer portion of the media were circular with a few interspersed longitudinal fibers. There was no collagen in the intima and the small amount which was present in the media lay near the adventitial border.

The ductus arteriosi of seven fetuses varying from 33 to 45 cm. in length showed slight changes from the 28 cm. fetuses. The lumina remained widely patent. The intimal mounds, which continued to be composed of elastic fibers and smooth muscle, showed a definite but variable degree of enlargement in respect to their height and width. Beneath the intimal mounds the tendency to fragmentation of the internal elastic lamina was more marked. The media showed no structural change from the previous specimens.

Five ductus arteriosi taken from full-term infants, ranging from a stillborn infant to those living 3 days, showed very little change. The intimal mounds were slightly larger, contained a moderate amount of elastic tissue and in several instances contained also a slight amount of collagen. The media remained loose and thin, with the muscle fibers coursing in the fashion previously described. In the midportion of the media of several of these specimens there were cellular whorls which were composed predominantly of concentric layers of smooth muscle fibers.

Four specimens obtained from infants living 4 days showed minor changes from those previously mentioned and in addition showed variations among themselves. The lumina of three were widely patent, whereas the lumen of the fourth was completely occluded by an early

organizing thrombus. The intimal mounds were variable in size but seemed slightly larger than in younger specimens and in three instances contained small amounts of collagen. Two of these ductus arteriosi were striking in that there were caps of cellular connective tissue projecting into the lumen from the surfaces of the mounds. These caps, which were subendothelial, apparently arise from an outgrowth of the connective tissue of the intimal mounds and can be differentiated readily from the intimal mounds because of the absence of elastic fibers in the caps. No changes in the structure of the media were observed.

The ductus arteriosi of six infants varying from 13 to 21 days of age showed progressive changes toward obliteration of the lumen. The lumen of one was occluded by an organizing thrombus. In this same specimen there was hemorrhage into the intima with extension into the media. In an infant, 21 days old, the lumen of the ductus arteriosus was occluded by vascular connective tissue, suggesting fusion of expanding connective tissue caps, although a revascularized organized thrombus could not be excluded. In three cases the lumina were markedly narrow and in one the lumen was widely patent. The intimal mounds present in this group were rich in elastic tissue and contained an increasing amount of collagen. The internal elastic lamina was maintained except at the mounds where it was fragmented. The media was more cellular, slightly thicker and more compact with an increase in elastic tissue and collagen.

There was anatomical closure of four ductus arteriosi taken from infants varying from 22 to 30 days of age. In several, a slitlike orifice persisted in the central portion while one or both ends were occluded by expansion of the intimal mounds. In two the central portion was occluded by organizing thrombi. In all the intimal mounds were large, containing variable amounts of collagen and elastic tissue. Degenerative changes, as evidenced by acellular pink-staining material rich in elastic fibers, were present in the intimal mounds of two specimens. The media was similar to that of the previous group.

From the period of 1 to 3 months of age, six ductus arteriosi were studied. In five the lumina were occluded. This occlusion was effected largely by a proliferation of the intimal mounds with or without overlying caps of loose connective tissue. In one instance the lumen was occluded by an organized thrombus, while another had an unorganized thrombus in the lumen with hemorrhage into an intimal mound. In an infant, 3 months old, the lumen of the ductus arteriosus was patent but narrowed to a small slit throughout its course. The intimal mounds in all were increasingly rich in elastic tissue and contained variable, often

large, amounts of collagen. Smooth muscle fibers became less conspicuous in the intimal mounds. No degenerative changes were present in the specimens in this group. The internal elastic lamina was frequently obscured by the abundance of elastic fibers in the intima and media. The media was dense, thick and rich in elastic fibers. With the increased thickness of the media the muscle fibers were almost entirely circular, with disappearance of the abundant longitudinal and radial fibers which were observed in earlier groups.

The ductus arteriosi of ten infants from 3½ months to 1 year of age were examined. In every instance the lumen was occluded. The obliterated lumen contained a sparsely cellular tissue rich in collagen and elastic fibers. At 5 months of age degenerative changes were observed in the intima. By 7 months of age the lumen of the ductus arteriosus was occluded by acellular hyalinized material rich in elastic tissue. There was a variable degree of vascularization in the obliterated lumina. The ductus arteriosus of one infant, 6 months old, showed degenerative changes below the internal elastic lamina or in the inner portion of the media. The media otherwise showed no significant change from the previous group.

In a series of eight specimens taken from children varying from 1 to 7 years of age the lumina were occluded in five. In one child, 14 months old, and in another, 2 years old, the lumina of the ductus arteriosi were patent but persisted only as narrow slits. The lumen of a specimen obtained from a child of 4 years contained an unorganized thrombus. Degenerative changes in the vessel wall were more marked than previously. The media was dense and rich in collagen and elastic tissue. In this group, as in some of the older groups, there was occasionally a dense, thick elastic membrane lining a slitlike lumen. Further from the central axis there was often a second incomplete elastic lamina which probably represented the remaining fragments of the original internal elastic lamina.

Sixteen ductus arteriosi were obtained from adults varying from 16 to 80 years of age. The central portion of a specimen from a young adult, 16 years old, was occluded by an organizing thrombus while the pulmonic and aortic portions were completely obliterated. In two adults, 18 and 20 years old, small slitlike lumina persisted throughout the course of the ductus. The remaining specimens were closed completely, or only at the pulmonic end as observed in two adults of 20 and 48 years of age. Degenerative changes were more marked, especially in elderly people where calcification, bone formation and cartilage were occasionally observed. The borderline between intima and media became lost and the entire structure was usually represented

by a mass of dense elastic tissue, collagen, hyaline material and a small amount of smooth muscle which lay toward the outer portion of the vessel wall. In longitudinal sections the intima of the pulmonary artery and aorta became thickened over the obliterated ends of the ductus arteriosus. This thickening, although less pronounced, was also present in younger age groups. It is at this site that atheromatous plaques are a common occurrence in the aortas of adults, even in the absence of marked atheromatous lesions elsewhere in the aorta.

Several unusual histological changes were observed in this whole series of ductus arteriosi. In the three patent ductus of the window type, there was, at the site of junction of the pulmonary artery and aorta, a slight thickening of the adjoining intimas, and a contrasting looseness of the media, with elastic tissue streaming from each major vessel toward the adventitial surface. The thickened intima contained elastic tissue, smooth muscle and, in one instance, collagen. There was no internal elastic lamina at the site of junction.

In four instances dissecting aneurysms were observed in the midportions of the cylindrical ductus arteriosus. Two specimens, which were from twins, 45 cm. in length and living 12 and 14 hours respectively, showed fresh hemorrhage in the inner and midportion of the media with elevation of the intima. The pulmonic and aortic ends were not involved. Vascular structure was otherwise similar to that previously described in this age group. One specimen from a full-term infant surviving for 12 hours showed mucinous degeneration with cyst formation in the midportion and outer portion of the media with recent hemorrhage involving half the circumference of the vessel. In an infant, 15 days old, the ductus arteriosus showed a hemorrhage extending directly from the lumen into the outer half of the media. In none of these four was there any evidence of rupture into the adventitia or any evidence of extension into the adjoining great vessels.

Summary and Conclusions

From a series of ductus arteriosi obtained from 71 autopsies, giving a range from 28 cm. fetuses to individuals 80 years of age, the mechanism of closure was studied. In a 28 cm. fetus the ductus arteriosus has a well defined internal elastic lamina which is subendothelial, except in several areas where there are low intimal mounds projecting into the lumen, rendering it eccentric. The mounds, which are composed of smooth muscle and fine elastic fibers, appear to arise from the internal elastic lamina which has a tendency to fragment beneath them. The media is loose in structure and is composed of fine, wavy elastic fibers and smooth muscle fibers. This affords a striking contrast

to the aorta and pulmonary artery where the media is compact and composed of laminated bundles of dense elastic fibers. In the ductus arteriosi of full-term infants the only variation from this pattern lies in the slight increase in size and number of the intimal mounds.

Anatomical closure is effected largely by an increase in size and perhaps in number of these intimal mounds, which gradually become infiltrated with collagen. Frequently, subendothelial caps of loose connective tissue grow from the surfaces of the intimal mounds and assist in the obliterative process.

During, and subsequent to, the process of obliteration of the lumen the intima gradually becomes more rich in elastic tissue. The media becomes denser, thicker, and richer in collagen and elastic tissue. The internal elastic lamina usually becomes obscured by the dense elastic tissue of the intima and media. Degenerative changes appear in the vessel wall and in the obliterated lumen as early as 5 months of age. Anatomical closure of the lumen of at least a portion of the course of the vessel is usually effected by the third or fourth week of life. A small, slitlike, microscopical lumen may persist for several months or occasionally longer. It appears that the central portion of the ductus arteriosus remains histologically patent longer than the aortic and pulmonic ends. In a few instances the pulmonic end is the first portion to become occluded. Thrombosis may occur in the lumen at any time from 4 to 30 days of age but has been observed as late as 4 years of age in this series. This is not a constant finding. In older age groups the ductus arteriosus consists of a dense mass of collagen and elastic tissue with only a few remaining muscle fibers. Hyalinization, calcification and even cartilage formation occur in this group.

It is apparent from this series of ductus arteriosi that many variations in histological structure from this described pattern may occur. These variations may in part account for the seemingly contradictory observations of other investigators.

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DESCRIPTION OF PLATES

PLATE 94

FIG. 1. Fetus 28 cm. in length. Low intimal mounds with fragmentation of the internal elastic lamina. Media loose, and poor in elastic tissue. Elastic tissue stain. × 105.

FIG. 2. Full-term fetus. Fragmentation of elastic lamina at base of intimal mound. Loose structure of media. Elastic tissue stain. × 185.

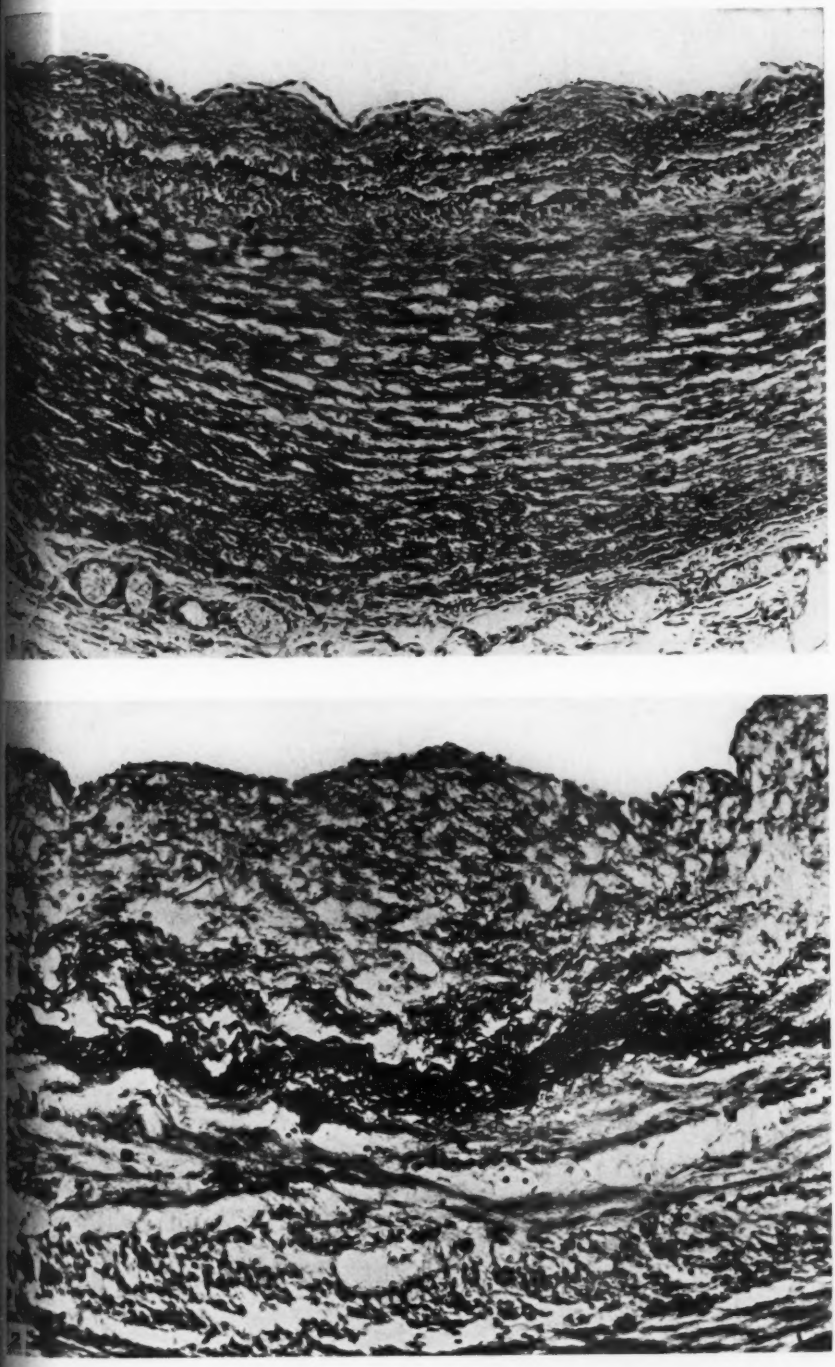
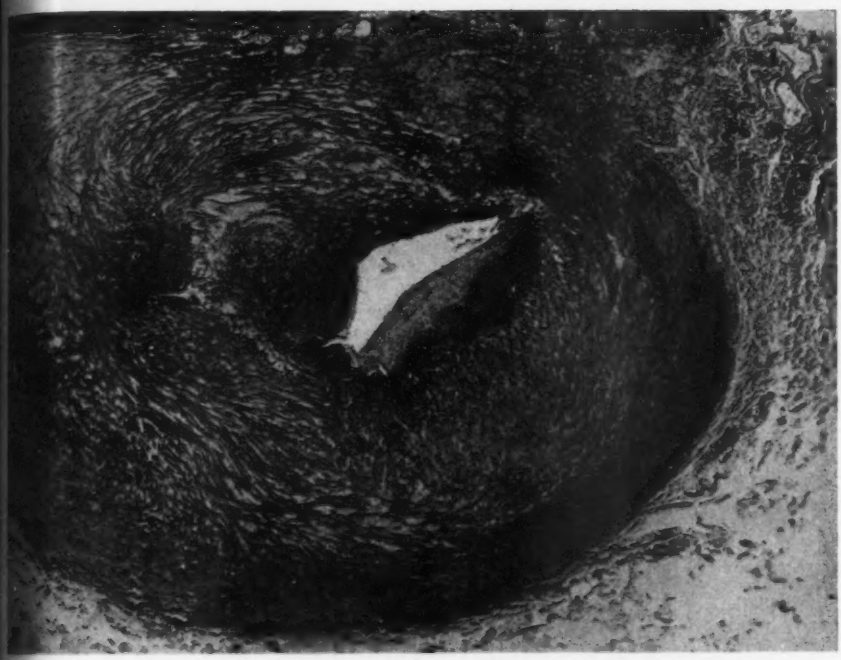


PLATE 95

FIG. 3. Infant, 13 days of age. Intimal mounds of increased size crowned by connective tissue cap. The media is less loose in structure. Elastic tissue stain. × 35.

FIG. 4. Infant, 21 days of age. Lumen obstructed by connective tissue, suggesting an organized, recanalized thrombus. Increase in amount of elastic tissue of media. Elastic tissue stain. × 80.



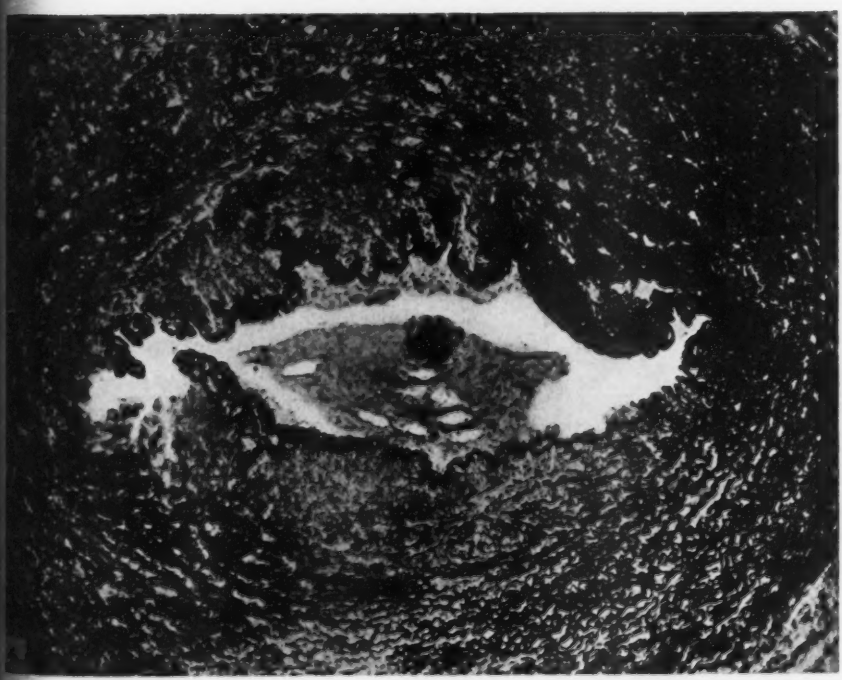
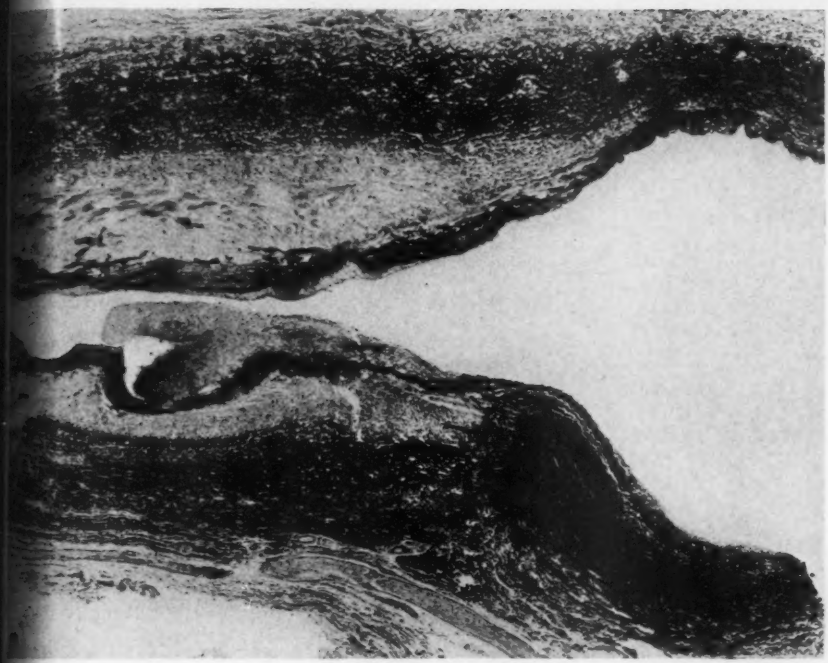


PLATE 96

FIG. 5. Infant, 22 days of age. Longitudinal section of obliterating aortic orifice of the ductus arteriosus showing aortic wall rich in elastic tissue while the ductus arteriosus is comparatively poor in elastic tissue and of looser structure. The streaming-in of elastic tissue, chiefly to the outer portion of the media, may be seen, but a slight amount is divided toward the intima to form the internal elastic lamina. Elastic tissue stain. × 25.

FIG. 6. Infant, 2 months of age. Longitudinal section of the obliterated aortic orifice of the ductus arteriosus with intimal connective tissue thickening at the site. × 35.



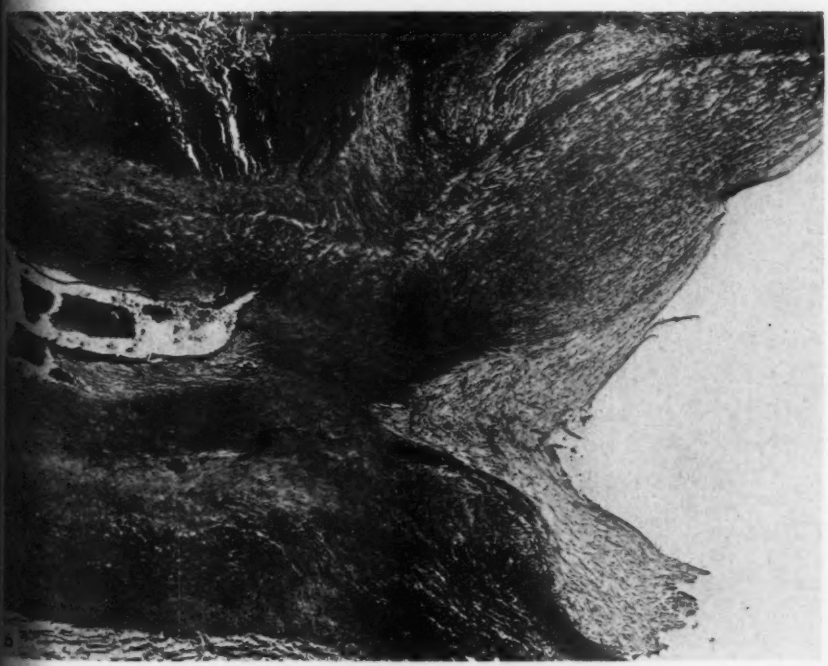
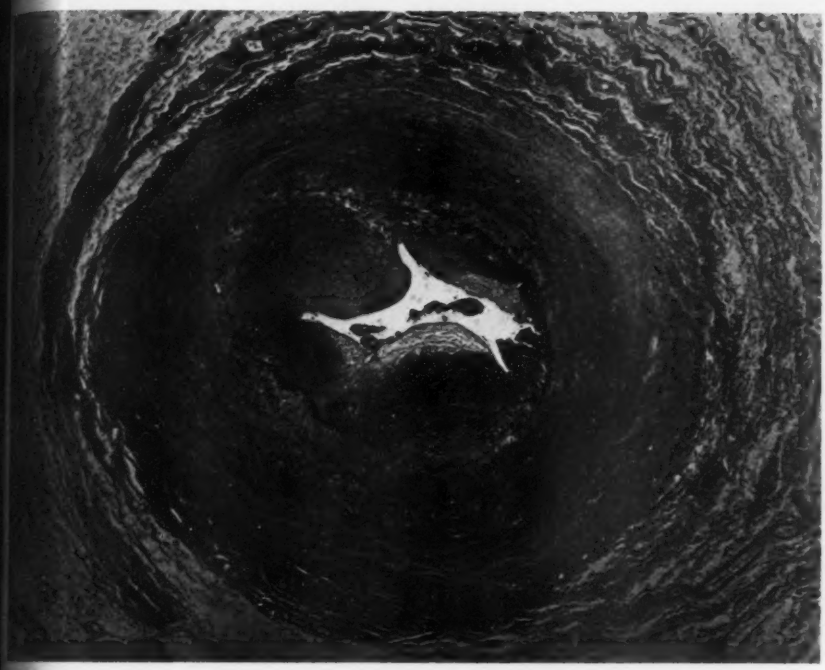
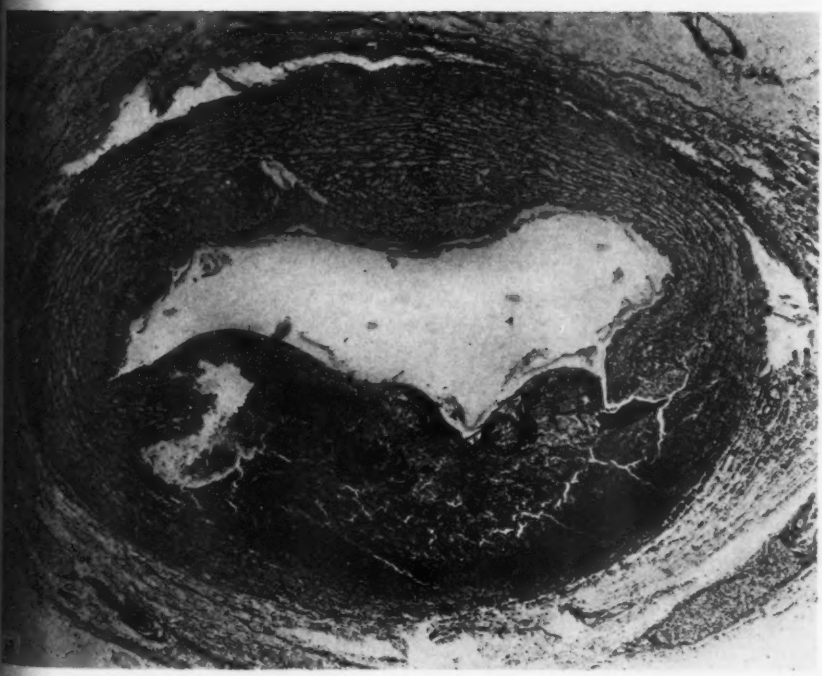


PLATE 97

FIG. 7. Infant, 6 months of age. Marked degenerative changes. The internal elastic lamina remains distinct. The intimal mounds are now composed of collagen, connective and elastic tissue. Elastic tissue stain. × 35.

FIG. 8. Newborn infant. Dissecting aneurysm of the ductus arteriosus. Intimal mounds present, but not prominent. Hematoxylin and eosin stain. × 30.





TWO ANTAGONISTIC EFFECTS OF UNDERFEEDING ON THE ADRENAL CORTEX OF THE GUINEA PIG *

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As early as 1892, Martinotti [1] noted that when guinea pigs were starved for 3 or 4 days, the number of mitoses in the adrenal cortex increased from the usual 3 or 4 mitoses per section to as many as 20 to 25 per section; while the large majority of the mitotic figures under these conditions were normal, some seemed to be abnormal in character. Subsequently, however, Bonnamour [2] found no increase in mitotic activity in the suprarenal cortical cells of rats, guinea pigs, rabbits or cats when these animals were starved to such an extent that they died. Rondoni and Montagnini [3] also were unable to find any increase in mitotic activity in the adrenals of starved guinea pigs, and Jackson [4] similarly reported negative results in studying the adrenals of underfed rats. These were, to our knowledge, the only investigations in which the degree of proliferation of adrenal cortical cells was actually determined, and, as stated, all the more recent publications indicated that there was no increase in mitoses in the adrenal cortex of undernourished guinea pigs. However, an observation in humans apparently substantiates Martinotti's findings. Byrne [5] noted that the adrenals of soldiers who had died from underfeeding in German prison camps during the first World War were enlarged to 1.5 times the normal size; the enlargement, from the naked-eye appearance, appeared to be mostly cortical. Microscopic evidence of proliferation of the cells of the adrenal cortex was not recorded and it is not admissible to use the increased thickness of the adrenal cortex as a criterion of active growth processes, because such a change might depend upon the infiltration of the cortical cells with fatty substances or with carbohydrates.

A number of years ago it was noted by us that when guinea pigs were markedly underfed, the cells of the adrenal cortex became enlarged and there was an extraordinary increase in the number of mitotic figures. Approximate counts showed 100 or more mitoses in a section. In some of the mitoses the chromosomes became fragmented after reaching the metaphase. However, changes were not noted in all

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experiments and for this reason publication of these experiments was deferred. It was in order to clear up, if possible, these variations in the results that the following investigations were undertaken.

Experiments

Seventy-two male and female guinea pigs, as a rule ranging in weight from 190 to 250 gm., but occasionally weighing as much as 250 to 300 gm., were used in additional experiments. These animals were fed a diet consisting of rabbit chow and greens, mixed in the usual proportions and containing the essential vitamins as well as carbohydrate, fat and protein, but they received diminished amounts of this feed for periods ranging from 2 to 20 days. The guinea pigs were weighed each morning and the quantity of food for any given day was regulated in accordance with the loss of weight of each animal during the previous 24 hours. Certain groups were underfed to a greater degree than others, as indicated in Tables I to IV. In one experiment, 10 male guinea pigs were underfed for 14 days and then refed for periods varying between 2 and 8 days, in order to determine whether the changes which occur in the adrenal cortex as a result of underfeeding are reversible. Mitotic counts were made in the manner described in a previous publication;[6] the average number of mitoses per section was determined by counting 25 sections through the central half of the adrenal gland.

In Table I are shown the results of mitotic counts of the adrenal cortex in 23 underfed male guinea pigs whose weight loss ranged between 20 and 55 grams. The controls for this experiment consisted of 37 normally fed male guinea pigs, selected in such a manner that their weights ranged from that of the heaviest animal at the onset of underfeeding (300 gm.) to that of the lightest guinea pig at the completion of the period of underfeeding (150 gm.). The average number of mitoses per section in these 37 controls was 3.2. It can be seen that as little as 2 days of marked underfeeding results in an elevation of the number of mitoses. In the 7 male guinea pigs underfed for 2 and 4 days, the average mitotic count was 10.2, with a range of variation between 5.1 and 18.1. The average weight loss in these guinea pigs was 31.4 gm. After 7 days of underfeeding the average mitotic count for 8 male guinea pigs was only slightly higher than after 2 to 4 days of inanition (10.4 mitoses per section), and the average weight loss was also somewhat higher, being, on the average, 40.4 gm. After 10 to 12 days of underfeeding the average mitotic count was 9.3 and the average weight loss was 38.8 gm., while after 15 days of underfeeding the average number of mitoses was only slightly above that of

the controls (3.8 mitoses per section); the average weight loss at this time was 42.5 gm.

From these experiments it may be concluded that the mitotic count was increased as early as 2 days after the beginning of the underfeeding and continued to be elevated to approximately the same level for about 12 days of inanition. After 15 days of underfeeding the

TABLE I

The Effect of Severe Underfeeding on Mitotic Activity in the Adrenal Cortex of Male Guinea Pigs

Guinea pig no.	Period of underfeeding (days)	Total weight loss (gm.)	No. of mitoses per section*
1	2	25.0	13.1
2	2	20.0	6.2
3	4	30.0	5.2
4	4	55.0	18.1
5	4	25.0	8.6
6	4	25.0	15.0
7	4	40.0	5.1
		31.4 average	10.2 average
8	7	50.0	12.0
9	7	50.0	10.1
10	7	40.0	20.9
11	7	52.0	10.3
12	7	42.0	9.6
13	7	33.0	5.6
14	7	28.0	7.3
15	7	28.0	7.2
		40.4 average	10.4 average
16	10	30.0	4.4
17	10	50.0	17.5
18	12	35.0	4.4
19	12	40.0	10.9
		38.8 average	9.3 average
20	15	45.0	2.7
21	15	40.0	2.8
22	15	35.0	7.3
23	15	50.0	2.5
		42.5 average	3.8 average

* In 37 male guinea pigs used as controls, weighing 150 to 300 gm., the average number of mitoses per section was 3.2.

count had returned almost to normal. However, the cortex of guinea pigs markedly underfed for 15 days is not normal in appearance, and the decrease in mitotic count may be due to certain degenerative changes which have occurred at this time, rather than to a return to the normal state of the gland. The microscopic changes in cellular structure will be discussed in another part of this paper. Furthermore, the decrease in the number of mitoses after 15 days of underfeeding

may be due in part to the fact that the loss of weight per unit of time has been less in these guinea pigs than in those examined at earlier periods.

In Table II are shown the results of an experiment in which male guinea pigs were underfed for 4 to 10 days, in such a manner that the loss in weight did not exceed 20 gm. In this experiment 14 guinea pigs lost an average weight of 14.3 gm. Fourteen normally fed male animals served as controls; they ranged in weight between 170 and 200 gm. and had an average mitotic count of 3.6. The average mitotic count in the 7 guinea pigs underfed from 4 to 7 days was 0.9, while in the

TABLE II

The Effect of Moderate Underfeeding on Mitotic Activity in the Adrenal Cortex of Male Guinea Pigs

Guinea pig no.	Period of underfeeding (days)	Total weight loss (gm.)	No. of mitoses per section*	
1	4	10.0	0.2	
2	7	5.0	0.8	
3	7	20.0	3.8	Average no.
4	7	10.0	0.8	of mitoses per
5	7	5.0	0	section, 0.9
6	7	15.0	0	
7	7	15.0	0.4	
8	10	15.0	4.0	
9	10	20.0	0	
10	10	20.0	1.8	Average no.
11	10	15.0	0.2	of mitoses per
12	10	20.0	0.8	section, 1.6
13	10	15.0	4.4	
14	10	15.0	0.2	
		14.3 average	1.2 average	

* In 14 male guinea pigs used as controls, weighing 170 to 200 gm., the average number of mitoses per section was 3.6.

7 males underfed for 10 days it was 1.6. The average number of mitoses per section in all 14 moderately underfed males was 1.2. It appears, therefore, that there is some depression of karyokinetic activity in guinea pigs underfed to a moderate degree; this depression seems to be somewhat greater during the first 7 days of underfeeding than after the 10-day period, as is evidenced by the fact that there were 6 animals in which there was less than one mitosis per section in the former group and only 4 such guinea pigs in the latter group. No marked histologic changes in the adrenal cortex were noted in this series.

Table III shows the results of counts carried out in markedly underfed female guinea pigs. The controls for this experiment were chosen in the same way as those for the previous two experiments and consisted of 43 female guinea pigs with an average count of 4.9 mitoses.

The degree of underfeeding in this experiment was similar to the corresponding experiment with male guinea pigs (Table I). It is evident from a comparison of the figures in Tables I and III that underfeeding causes a very much greater increase in mitoses in females than

TABLE III

The Effect of Severe Underfeeding on Mitotic Activity in the Adrenal Cortex of Female Guinea Pigs

Guinea pig no.	Period of underfeeding (days)	Total weight loss (gm.)	No. of mitoses per section*
1	4	37.0	14.9
2	4	37.0	22.8
		37.0 average	18.9 average
3	7	35.0	13.4
4	7	30.0	19.2
5	7	30.0	91.3
6	7	35.0	15.9
7	7	35.0	12.3
8	7	35.0	24.9
9	7	27.0	13.1
10	7	35.0	11.6
11	7	40.0	7.4
12	7	35.0	10.9
13	7	28.0	9.2
		33.2 average	20.8 average
14	10	25.0	109.0
15	10	25.0	22.0
16	10	30.0	21.1
17	10	25.0	56.9
		26.3 average	52.2 average
18	12	30.0	44.6
19	12	45.0	116.0
20	12	35.0	197.2
		36.7 average	119.3 average
21	15	50.0	3.0
22	15	35.0	10.8
23	15	85.0	11.4
24	15	80.0	11.2
		62.5 average	9.1 average
25	20	80.0	0

* In 43 female guinea pigs used as controls, weighing 150 to 350 gm., the average number of mitoses per section was 4.9.

in males. In 2 guinea pigs, underfed for 4 days, the average weight loss was 37 gm. and the average mitotic count was 18.9 per section, as compared with 10.2 mitoses per section in males underfed for a similar period. After 7 days of underfeeding the mitotic count in females rose on the average to 20.8 per section; the average weight loss of these 11

guinea pigs was 33.2 gm. The mitotic count in males underfed for a similar 7-day period was 10.4. In 4 female guinea pigs underfed for 10 days the average mitotic count rose to 52.3 and the average weight loss was 26.3 gm.; 3 females underfed for 12 days had, on the average, 119.3 mitoses per section and an average loss in weight of 36.7 gm. Males similarly underfed for 10 to 12 days showed an average of 9.3 mitoses per section. When the 7 female guinea pigs underfed for 10 and 12 days are combined into a single group, the average mitotic count is 81.0. Further, 4 females were underfed for 15 days and showed an average weight loss of 62.5 gm.; the average mitotic count for this group was 9.1 per section. In males markedly underfed for the same length of time the mitotic count was 3.8 per section. Finally, after 20 days of underfeeding, 1 guinea pig had lost 80 gm. in weight and had a mitotic count of 0. Here also, as in the experiment in which marked inanition was produced in males, the return of mitotic proliferation to a low state of activity does not indicate a return to the normal state of the adrenal cortex, but rather to a state of exhaustion of the adrenal cortical cells. It can be seen from this experiment that while underfeeding causes a much greater rise in mitoses in females than in males, the character of the curves representing the changes in mitotic activity as a function of time are somewhat similar in the two sexes.

In a fourth experiment (Table IV) we attempted to ascertain whether the changes in the adrenal cortex resulting from marked underfeeding were reversible. Ten male guinea pigs were underfed for 14 days. Two of these animals were autopsied as controls at the end of this period of inanition; they had lost, on the average, 62.5 gm. in weight and showed a mitotic count of 3.4 per section. Two of the guinea pigs were refed for 2 days; one of these regained 15 gm. of its lost weight and had a mitotic count of 13.7, while the other regained only 5 gm. and had a mitotic count of 4.4. The average count for these two animals was 9.1. Two other males were refed for 4 days and regained 30 and 35 gm. respectively; their mitotic count was, on the average, 21.1. Two guinea pigs refed for 6 days regained 60 and 40 gm. of their original loss in weight and showed a mitotic count of 5.6 and 14.3 respectively; the average mitotic count for these two animals was 10.0 per section. Finally, two males were refed for 8 days, at which time they had regained completely their original loss in weight, as well as an additional 10 gm. The average number of mitoses in these two guinea pigs was 3.3.

From this experiment, it may be concluded that when male guinea pigs weighing about 250 gm. are underfed for a period of 14 days, in

such a way that they lose approximately 60 gm. (about 25 per cent of their original weight), the adrenal cortex shows a low mitotic count and manifests signs of exhaustion. When these animals are subsequently refed, the cortical cells go through a process of recovery characterized at first by increased mitotic activity. This increase in mitotic proliferation was at its height when the animals had regained approximately 50 per cent of their original weight loss. It was first noticed when 25 per cent of the lost weight was recovered, and it disappeared after about 80 per cent of the original loss in weight had been regained.

TABLE IV

The Effect of Underfeeding and Subsequent Refeeding on Mitotic Proliferation in the Adrenal Cortex of Male Guinea Pigs

Guinea pig no.	No. of days refed	Weight lost after 14 days of underfeeding (gm.)	Weight regained after refeeding (gm.)	No. of mitoses per section
1	0	60		4.3
2	0	65		2.5
				3.4 average
3	2	60	15 (25%)	13.7
4	2	50	5 (10%)	4.4
				9.1 average
5	4	60	30 (50%)	16.7
6	4	65	35 (53.8%)	25.4
				21.1 average
7	6	70	60 (85.7%)	5.6
8	6	50	40 (80%)	14.3
				10.0 average
9	8	50	60 (120%)	2.3
10	8	40	50 (125%)	4.2
				3.3 average

HISTOLOGIC CHANGES IN THE ADRENAL CORTEX IN UNDERFED GUINEA PIGS

The histologic changes which were noted in guinea pigs subjected to underfeeding are shown in Figures 1 to 4. In the adrenals of normal guinea pigs, a representative specimen of which is shown in Figure 1, the cortical cells in the outer third of the zona fasciculata contained a lipoid or fatty substance, which appeared as large vacuoles when the tissue was prepared by the paraffin technic. These vacuoles occupied a large portion of the cell and caused a compression and reduction in the size of the nucleus. When guinea pigs weighing 200 to 250 gm.

were underfed sufficiently to bring about a 15 to 20 per cent loss in weight in a period of 2 to 4 days, these vacuoles began to disappear and the cortical cells enlarged. The nuclei also increased in volume and became well rounded and vesicular. At this time mitotic figures appeared in increased numbers. Some mitotic divisions occurring in a single field are shown in Figure 2, which represents this stage of the process of inanition; in this figure only one vacuole can be seen. In Figure 3 is shown the condition which was present when the state of undernourishment was maintained for a period of 10 days. The vacuoles have entirely disappeared, the cortical cells and their nuclei continue to be enlarged and mitoses are numerous.

During the process of marked inanition this fatty or lipoid material disappeared as mitotic activity increased. In males, a few vacuoles usually persisted, but in females they were usually absent when mitotic activity was at a maximum. This finding corresponds to the difference in the degree of mitotic activity observed in the two sexes. In guinea pigs which were only moderately underfed, so that the loss in weight in the first 10 days ranged between 5 and 20 gm., the number of mitoses, instead of increasing, showed a definite decrease. In accordance with expectations, the vacuoles did not disappear from the cortical cells. In this case, therefore, the pure effect of underfeeding predominated, with a resulting decrease in proliferative activity. In male guinea pigs which were underfed and subsequently refed there was, at first, a complete absence of vacuoles after 14 days of underfeeding and the cells showed signs of exhaustion, as described below. With refeeding, there was a lag period of about 4 days before vacuolar inclusions were noted, and in the period from 4 to 6 days, although present, they were in smaller numbers and of diminished size as compared with normally fed guinea pigs. After 8 days of refeeding, the normal number of vacuoles reappeared and their size also increased; accordingly, there was no longer any increase in the number of mitoses. From these observations it may be concluded that a direct relation exists between the increase in size of cells and augmented mitotic activity on the one hand, and the disappearance of fatty substances on the other hand.

When marked inanition was maintained for about 15 days, signs of exhaustion set in. The vacuoles were absent but the cells of the zona reticularis and deeper zona fasciculata underwent degeneration, the nuclei becoming either pyknotic or karyorrhetic and many of the cells disintegrated completely. The more peripheral cells of the zona fasciculata were swollen and contained a frothy albuminous material, while the nuclei were compressed and pyknotic. At this stage only

the cells of the zona glomerulosa and some of the outermost cells of the zona fasciculata showed little evidence of damage, and it is probably from these cells that the cortex regenerates when the process of refeeding is instituted. This state of exhaustion occurs earlier in males than in females; in certain males it was observed as early as after 12 days of underfeeding, whereas in females it was often just beginning to appear after 15 days of underfeeding, when the mitotic count in some of these animals was still somewhat elevated.

Finally, it was noted that in many of the cells undergoing mitotic division this process did not progress beyond the metaphase. In these cells the chromosomes frequently became fragmented and particles of chromatin of various sizes and shapes were spread haphazardly throughout the cytoplasm. When mitotic activity was at a maximum, as many as 20 to 30 per cent of the cells undergoing karyokinesis showed this dispersed type of chromatin. However, even if these cells were omitted from the mitotic counts, the latter would still be considerably elevated.

Discussion

These experiments may account for the differences in the results obtained in some previous investigations. Thus, the very high degree of proliferation, the frequent occurrence of abnormal mitoses in some cases and the lack of these changes in others may have been due to differences between male and female guinea pigs or to differences in the length of time during which the animals were exposed to underfeeding. These factors may explain the lower counts found by Martinotti.[1] Moreover, Martinotti did not state to what degree his animals were underfed; on the other hand, Bonnamour,[2] as well as Rondoni and Montagnini,[3] apparently starved guinea pigs to such an extent that the adrenal glands were in a state of exhaustion at the time of examination.

The explanation of the failure of Jackson[4] to find an increase in proliferative activity in the rat as a result of inanition may be due to factors other than the degree of underfeeding, since this investigator produced undernourished states of varying degrees and for varying periods of time. However, the responsiveness of various endocrine glands is different in the rat than in the guinea pig. Thus Loeb[7] and Loeb and Bassett,[8] as well as Thurston,[9] have shown that the thyroid gland of the rat responds much less actively to the stimulating effects of anterior pituitary extract than does this gland in the guinea pig, and in some unpublished experiments Blumenthal has observed that the adrenal cortex of the rat likewise responds to a lesser degree than

does the adrenal of the guinea pig, when animals of these two species receive equal amounts of acid extract of bovine anterior hypophysis per unit of weight. Further differences in the mode of reaction of the adrenal glands of these two species are indicated by the fact that bacterial toxins and particularly diphtherial toxin, which produce marked pathologic changes in the adrenal cortex of the guinea pig, have no grave effect on this gland in the rat.

As to the mechanism underlying the remarkable increase in proliferation in the adrenal cortex caused by undernourishment under certain conditions, the following facts have to be considered:

(1) Loss of weight of organs, if it reaches a certain degree, has the tendency to lower the proliferative activity of organs and tissues; moreover, it tends to inhibit the increase caused by some stimulating factors. This is exemplified by the behavior of the thyroid gland, where undernourishment decreases the number of mitoses occurring in normal organs and where it diminishes or prevents also the increase in mitoses usually caused by administration of potassium iodide, as shown by Gray and Loeb [10] and Rabinovitch,[11] as well as by Rabinovitch and Gray.[12] However, the intensity of the effect of undernourishment on the proliferative activity varies in different organs and tissues; it is less in the epidermis than in the thyroid of the guinea pig (Loeb, Haven, Genther and Friedman [13]). In no organ other than the adrenal has it so far been observed that undernourishment causes an increase in the number of mitoses.

(2) The proliferative activity of tissues is strongly influenced by certain secondary differentiations, which take place either in the course of embryonal development or by changes which occur in the connective tissue during extra-uterine life. The latter are associated with the production of paraplastic structures and the deposit of various substances within the cells composing these tissues. Thus the production of polymorphonuclear leukocytes is connected with a cessation of mitoses. In the epidermis, processes which precede keratinization likewise cause a cessation of mitotic activity; stimulation of such cells may lead to a production of amitoses instead of mitoses. In the guinea pig, the transformation which the granulosa cells of the ovarian follicles undergo during the process of maturation, consisting in an increase in cytoplasm which stains strongly with eosin, is associated with a cessation of proliferative activity, which can, to a certain extent, be resumed when the mature granulosa cells become converted into corpus luteum cells (Loeb [14]). Very instructive in this respect is the behavior of the mammary gland of the mouse. Here, under the influence of certain hormones, proliferation takes place, as is indicated by an increase

in mitoses. But, if fat droplets are deposited in these cells in the process of milk production, mitoses cease, as a rule; they are found usually only in cells in which these droplets are as yet lacking. It is known that in the cortex of the adrenal gland fatty and lipoid substances, and to a less extent also glycogen, are found in quantities which vary under different conditions. Considering all these facts, it is reasonable to assume that it may be the deposit of certain fatty or lipoid substances in the cortical cells which diminishes the mitotic proliferation, and that removal of these substances might lead to an increase in mitoses. The observations which we have recorded here support this interpretation of the effect of undernourishment in cell proliferation in the adrenal cortex. Whenever a marked increase in the number of mitoses occurs in the adrenal cortex, fat or lipoid vacuoles have disappeared in the cortical cells in which proliferation may take place. Moreover, the differences in the degree of mitotic activity which are found in underfed male and female guinea pigs are in agreement with this interpretation. The deposit of this substance in the cells brings about changes unfavorable to mitotic proliferation, which otherwise would occur under the influence of stimulating hormones. But in addition to this specific factor, the general effect of undernourishment, consisting in a diminution or cessation of mitotic activity, is also found. If the undernourishment exceeds a certain degree or extends over a longer time, the number of mitoses in the cortical cells diminishes again, or ceases altogether. Then if a refeeding occurs, there may be observed a stage when the injurious effect of undernourishment is removed and the cell is ready to respond again to hormonal stimulation with an increased mitotic proliferation, a period which will be terminated by the reappearance of fatty deposits or perhaps also under the influence of some other changes. As to the abnormal character of some of the mitoses which appear in undernourished guinea pigs, it may be suggested that this is due to the combined effect of the removal of the fatty cell-inclusions, which is favorable to the development of mitoses, and of the injurious action which starvation otherwise exerts on the cell metabolism; presumably the deficiencies due to starvation of the cells cause degenerative conditions during the latter stages of the mitoses.

In experiments which one of us is at present carrying out, it has been noted that when acid extract of bovine anterior hypophysis is administered to guinea pigs, there is a marked loss of fat vacuoles with a coincident increase in the size of the cortical cells and a marked rise in mitotic activity. Also, when mitotic proliferation is very pronounced, abnormal mitoses, with fragmentation of chromatin, similar to that

which has been described in these investigations, are observed. When this extract is administered to older guinea pigs, whose adrenal cortical cells contain a greater number of these fatty inclusions than those of younger animals and in whose cells other paraplastic substances such as pigments may be present, the degree of mitotic response to hypophyseal extract is markedly diminished. The difference in the degree of proliferative response to underfeeding in male and female guinea pigs may perhaps be due to differences in their ability to metabolize these lipoid or fatty substances.

It thus appears probable that the presence of certain fatty or lipoid substances in cortical cells inhibits their proliferation, which otherwise would take place under the influence of hormones, and that, conversely, the discharge of lipoid or fatty materials from the adrenal cortex may lead to an increase in mitotic activity on the part of the cells of this gland. That this interpretation is correct seems more probable than the assumption that the changes in the fat or lipoid content are merely a concomitant of the increase in mitotic activity, but not its direct cause. Such an assumption is unlikely in view of the general parallelism between the elimination of these paraplastic substances and the increase in mitotic proliferation, and in view also of other factors which point to an inactivating effect of cell differentiation and of intracellular accumulation of paraplastic substances as already discussed.

It may then be concluded that the observations reported by us in this paper agree with the interpretation which we have given. The theory here presented may explain also some observations of Selye,[15] who noted that during the first 24 hours of the so-called alarm reaction a marked hyperplasia of the adrenal cortex occurs, although it is not stated that this is due to an actual increase in the mitotic activity. The increase in the size or number of cortical cells is accompanied by a loss of lipoid inclusions in the cortical cells, and in this case, too, the removal of these cell inclusions may be the cause of the increase in the number of cells.

Summary

1. Marked inanition produces an increase in mitotic activity of the adrenal cortical cells, which in males is most pronounced between the fourth and seventh days of underfeeding; the increase in mitoses is much greater in females than in males; the maximum in the former is reached after 10 to 12 days of undernourishment, when the number of mitoses per section may exceed 100.

2. The microscopic changes in the adrenal cortex, due to underfeeding, are characterized by a marked decrease in fatty or lipoid

vacuoles, an increase in the size of the cortical cells and the appearance of abnormal mitotic figures in which there is a dispersion of chromatin after the metaphase has been reached. The disappearance of fat vacuoles in female guinea pigs is practically complete when mitotic activity is at a maximum, while in males there may be some remaining vacuoles at this time. Correspondingly, the degree of mitotic response to severe underfeeding is considerably greater in females than in males.

3. It has also been shown that these changes are completely reversible, since when male guinea pigs are underfed for a sufficiently long period to bring about a state of exhaustion in the adrenal cortex, subsequent refeeding results first in a gradual increase in the number of mitotic figures to above the normal, and this condition is followed by a return of the cortex to the normal histologic state with a normal mitotic count.

4. When guinea pigs are only slightly underfed for periods lasting from 4 to 10 days, there is a suppression of mitotic activity rather than the increased proliferative activity observed with more marked degrees of inanition.

5. Under all conditions observed so far there was an inverse relation between the frequency of fatty or lipoid inclusions in the cortical cells and the frequency of mitotic proliferation of these cells.

6. The various effects of underfeeding on the adrenal cortex of guinea pigs can be explained if we assume that the removal of lipoid or fatty cell-inclusions eliminates a factor which inhibits mitotic proliferation (which otherwise would take place under the influence of certain hormones) and that this removal is responsible for the marked increase in mitoses which is observed in these cells under certain conditions of underfeeding. The increase in mitosis in the adrenal cortex of underfed guinea pigs must therefore be regarded as an indirect effect. It must also be assumed that underfeeding as such has the opposite effect, inasmuch as it diminishes the proliferative activity. Under different conditions one or the other of these effects predominates.

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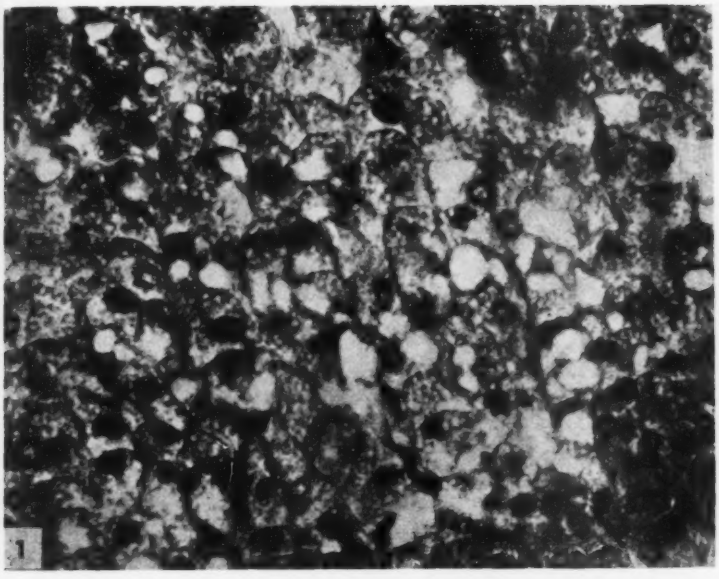
DESCRIPTION OF PLATES

PLATE 98

FIG. 1. Section of adrenal cortex of normal female guinea pig weighing 250 gm. Lipoid vacuoles are present in many of the cells of the outer zona fasciculata. There are no mitoses. × 440.

FIG. 2. Section of adrenal cortex of female guinea pig underfed for 4 days; weight loss was 40 gm. (from 245 to 205 gm.). The lipoid vacuoles have disappeared from all but one cell (v) in this region of the outer zona fasciculata. A number of mitotic figures are present; these are indicated by arrows. × 440.



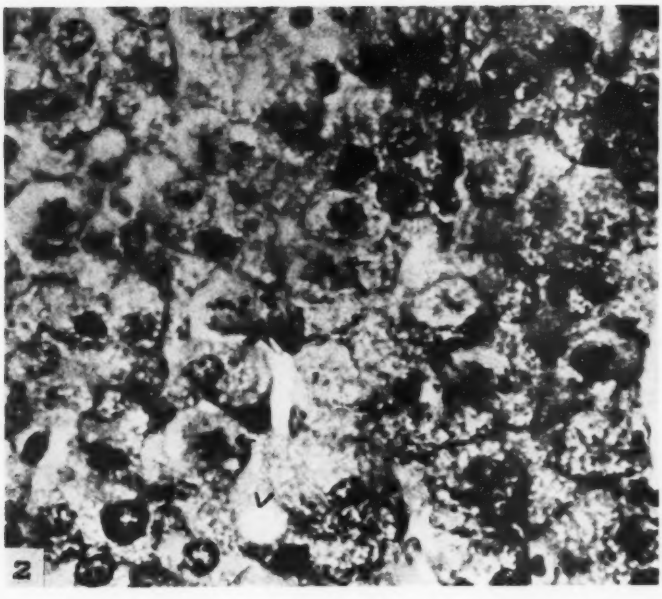


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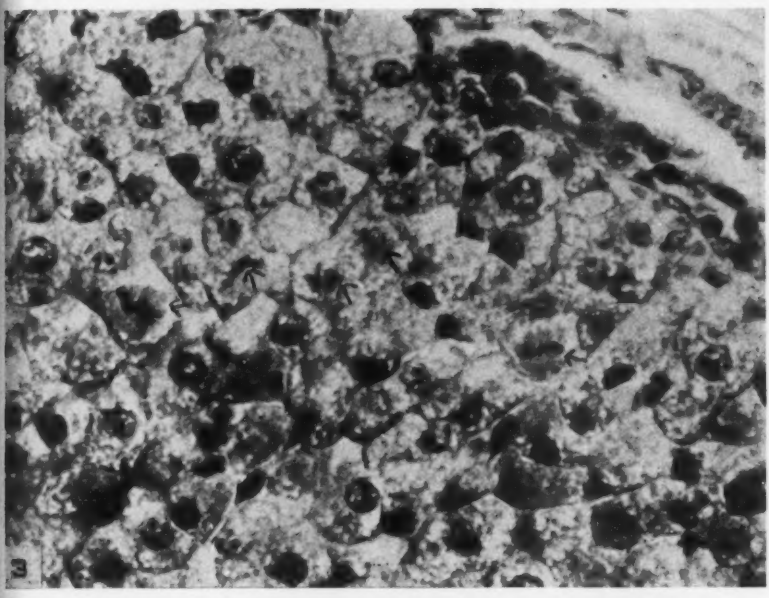
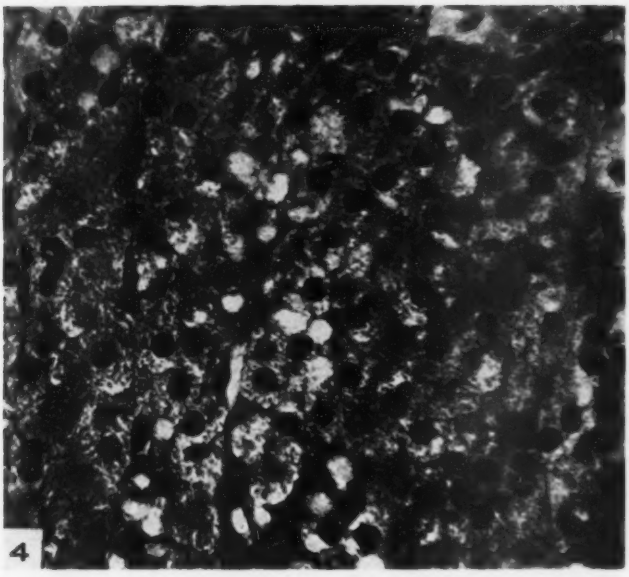
PLATE 99

FIG. 3. Section of adrenal cortex of female guinea pig underfed for 10 days; weight loss was 60 gm. (from 250 to 190 gm.). The lipoid vacuoles have completely disappeared from this region of the outer zona fasciculata. A number of mitotic figures are present; these are indicated by arrows. × 440.

FIG. 4. Section of adrenal cortex of a male guinea pig slightly underfed for 7 days; weight loss was 15 gm. (from 240 to 225 gm.). The lipoid vacuoles are still present in the outer zona fasciculata; no mitotic figures are visible and the cells and nuclei are somewhat smaller. × 440.





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FUNCTIONING ISLET CELL CARCINOMA WITH METASTASES TO LIVER *

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Spontaneous hypoglycemia has been established as usually secondary to islet cell tumors, either benign or malignant. Among these, the cases in which metastases have occurred have been surprisingly few. Frantz,[1] in a recent review of 96 tumors of islet cells, could find only 5 cases with proved metastases.[2-6] These are summarized in Table I. In addition, she listed 21 cases suspected of being malignant on the basis of capsule or blood-vessel invasion, but no report of metastases occurring in these patients has appeared in the literature to date (August 22, 1941).

Four additional cases,† including one case studied and autopsied in this hospital, of proved, functioning islet cell carcinoma with metastases have come to our attention. One was the recent case of Flinn, Beatty, Ginsberg and Hemsath,[8] giving a typical history of hypoglycemic attacks over a period of 5 months. Blood-sugar determinations as low as 20 mg. per cent were obtained. There was no operation. At autopsy an islet cell carcinoma was found in the head of the pancreas with metastases to the liver and lymph nodes. No assay of insulin was made. The pituitary was grossly normal. There were no other essential findings.

Jacobsen,[9] in a brief abstract, recorded in the history of a male, 36 years of age, that on the finding of glycosuria, a diagnosis of diabetes was made. On insulin regime he developed signs of shock with blood-sugar determinations as low as 25 mg. per cent. In the following year, the taking of sugar warded off attacks. At operation, an inoperable tumor, the size of a small grapefruit, replaced the head of the pancreas. Many nodules were seen in the liver. The diagnosis was first made from one of these. Biopsy showed a striking resemblance to islet cells, and beta granules were demonstrated. These findings were confirmed by autopsy. No assay of insulin was made. No photomicrographs were included as the case was to be reported in detail at a later date. We

* Received for publication, August 30, 1941.

† Not included among these is the case reported by Ballinger [7] of a tumor involving liver, right lung, right hilar lymph nodes, adrenal glands, spleen, kidneys, heart and a subcutaneous nodule. The pancreas showed no tumor. He interpreted the findings as an islet cell carcinoma arising in aberrant pancreatic tissue in the liver. There are so many peculiarities in the general picture that it is difficult to accept this case as one of proven islet cell carcinoma with metastases.

have reviewed the slides from this case and have confirmed the diagnosis (through the permission and kindness of Dr. V. C. Jacobsen).

Through the kindness and permission of Dr. Paul R. Cannon we have been able to review the slides and confirm the diagnosis of an additional case, the histological details of which are thus far unpublished although it has been mentioned in other reports.[10,11] A male, 36 years of age, had spontaneous hypoglycemic attacks for a few months. Fasting blood sugars as low as 16 and 23 mg. per cent were obtained. No glycosuria or ketonuria was observed. Intravenous glucose immediately stopped the attacks. The glucose requirement was 600 gm. daily. The body weight increased from 57.1 to 64.3 Kg. in 3 months. Exploratory laparotomy revealed many tumor nodules in the liver and a mass in the pancreas. Biopsy of a liver nodule was performed. At autopsy a carcinoma of the pancreatic islet cells was found, with metastases to the liver, peripancreatic, periaortic and mediastinal lymph nodes, one adrenal, spine, lungs and pleura. Assay of insulin was inconclusive.

Report of Case

F. H., a female patient, 48 years old, was studied in this hospital. Only a brief review of the clinical findings is given here, a more complete record being available.[12] The patient had been well up to June, 1935; at that time she began to suffer from occasional fainting spells and attacks of coma, lasting 12 to 48 hours, during which she would remain in a dazed and drowsy condition. During minor episodes, mental confusion, speech disturbances and blankness of vision were common. These attacks became more frequent until in 1937 she was having one or two a day. Attacks were very prone to occur in the early morning unless food had been taken at bedtime. A few lumps of sugar would sometimes ward off an attack. At the Worcester City Hospital, Worcester, Massachusetts, in June, 1938, the fasting blood sugar was as low as 27 mg. per cent. On a prescribed diet of high fat, low protein and carbohydrate, the patient did fairly well for a time. Then symptoms recurred, and in order to prevent an attack, feedings every 2 or 3 hours were required. On admission to the New England Deaconess Hospital on September 15, 1938, she was obese, weighing 191 pounds. Her usual weight in 1935 was believed to have been 135 pounds. General examination was negative. Fasting blood sugars varied from 30 to 50 mg. per cent. The glucose tolerance curve was characterized by a subnormal initial value, a normal course up to 3½ hours after the giving of glucose and a sharp falling away until 35 mg. per cent was reached at the end of 5 hours. A diagnosis of islet cell tumor was made. Operation was advised, and the patient went home promising to return for operation.

However, shortly after this she sustained an injury to her right ankle and remained in bed for 20 weeks. The attacks incident to hypoglycemia continued and for 2 weeks before readmission the attacks were very frequent, requiring feeding every 2½ hours. The patient did not return to the hospital until September 12, 1939, 1 year after her first admission. Examination showed extreme obesity, the weight now being 203 pounds, but no other abnormal findings were noted. Her blood sugar was low and it was unsafe to attempt fasting determinations. It was so difficult to control the hypoglycemia and the resulting stupor that it was decided to proceed at once with exploratory operation. On September 16, 1939, a laparotomy was performed by Drs. L. S. McKittrick and T. C. Pratt. This disclosed

a tumor in the tail of the pancreas with multiple nodules in the liver. The pancreatic tumor was excised and tissue for biopsy removed from one of the hepatic nodules. The postoperative course was complicated at first by bronchopneumonia; persistent purulent discharge from the pancreatic wound; thrombophlebitis and abscess of the right arm, probably associated with continuous intravenous therapy; marked anorexia; persistent and extraordinary shortness of breath, and rapid respiration as well as persistent high pulse rate. Physical examination and roentgenograms of the chest yielded no findings which entirely explained the cardio-respiratory findings. Finally, about 6 weeks after operation, she seemed to be improved and was allowed to sit in a chair for short periods. On November 3, 1939, she suddenly developed extreme dyspnea, rapidly became unconscious, pulseless and deeply cyanotic, without râles being heard in the chest, and died in 15 minutes.

The behavior of the blood sugar in the postoperative period merits attention. During the first week, on constant intravenous glucose, the values were at a high normal level (90 to 120 mg. per cent). When intravenous glucose was stopped, the values gradually increased to 250 mg. per cent, at which time glycosuria to the extent of 35 gm. per 24 hours was obtained. Protamine zinc insulin was administered for 1 month in dosage of 12 to 16 units before breakfast. With such treatment the glycosuria cleared up and the blood-sugar values were maintained at a satisfactory level. During the last 10 days of life no insulin was given, and on a carbohydrate intake of 150 to 200 gm. daily, blood sugar ranged from 110 to 150 mg. per cent.

Pathological Report Upon Surgical Specimen

(The following report was made by Dr. Shields Warren.) The surgical material consisted of a nodule from the pancreas and a specimen from the liver. Thin blocks were fixed in the following fluids: Zenker's, Helly's, and Bouin's as well as in a 4 per cent solution of formaldehyde and in absolute alcohol. Routine stains were made with eosin and methylene blue and phosphotungstic acid hematoxylin. Special stains were also used as indicated.

The pancreatic mass measured 1.8 by 1.8 by 1.3 cm. and was slightly irregular in outline with a rim of adherent pancreatic tissue. Microscopically the pancreatic tumor was composed of polyhedral cells arranged in various ways. Some grew in sheets which had the architectural relationship of islet cells. Elsewhere many elongated, irregular cells with almost clear cytoplasm tended to be arranged in rows with the long axes of the cells at a right angle to the row. They resembled one side of a long duct. These two types of cells merged into one another. More rarely, larger, cuboidal or columnar cells with almost clear cytoplasm tended to form acini and occasionally to secrete mucinous material. No cells resembling those in normal acini were seen. The nuclei of the three types were essentially similar, either round or oval, with finely divided chromatin. Mitoses were rare. The tumor cells penetrated through the capsule and invaded the surrounding pancreatic tissue. Blood-vessel invasion was not seen. Groups of tumor cells were widely separated by bands of dense, structureless hyaline ma-

terial. Special stains, especially Bensley's modification of Mallory's aniline blue stain [18] and Gomori's stain,[11] showed the bulk of the tumor to be made up of cells without specific granulation. However, rare, well defined beta cells were encountered, but no alpha cells. The adjacent normal pancreas provided an excellent control for the stains utilized.

The specimen from the liver measured 1.4 by 0.8 cm. and was made up of grayish pink tissue with a hard central focus. The histological structure was similar to that of the pancreatic tumor except that for the most part it was made up of central hyaline material with a few tumor cells at the periphery. No beta cells were demonstrated.

Postmortem Examination

Autopsy was performed 4 hours after death. The body was that of an obese, adult female, measuring 5 feet 2 inches in length. The anatomical diagnoses were: metastases of islet cell carcinoma in liver; thrombophlebitis of the right brachial, axillary, subclavian and internal jugular veins with complete occlusion; thrombosis with partial organization and occlusion of the superior vena cava propagating to the right auricle; massive pulmonary embolism; recent and old infarction of the right lung; marked pituitary basophilism; meningioma, 0.8 cm. in diameter.

The essential findings were: A sinus tract led from the skin surface to the region of the tail of the pancreas, but ended blindly in a smooth-walled pocket which did not communicate with the pancreas and contained amorphous material.

The *liver* weighed 1510 gm. Externally and on the cut surface it was studded with many firm, white, glistening, well demarcated nodules varying from 2 to 3 cm. in diameter. Those on the surface tended to be umbilicated. Many contained firm, hard, yellow centers. The tumor replaced one-third to one-half of the liver substance. Uninvolved portions of the liver were firm and reddish brown.

Microscopically, the liver nodules varied greatly in size and cellular content. The manner of growth resembled an islet on a large scale. There were three types of cells: (1) The cell type found most frequently was tall columnar, and such cells were aligned in long ribbonlike strands. The cytoplasm was generally acidophilic, but some foci showed a small amount of basophilic, finely granular material in one end of the cell. In other foci the spaces between the cells were filled with strands of amorphous material with a basophilic tinge suggesting thin mucus. (2) In other parts, tall, regular columnar cells formed tubes with basement membranes. The nucleus was at the membrane end of such cells, while the inner two-thirds was composed of clear, mucus-contain-

ing cytoplasm. In such places the tumor appeared to be attempting to form ducts. (3) In a few areas the cells were polyhedral in shape with centrally placed nuclei and acidophilic cytoplasm. Occasionally these cells were binucleate. Although many cells resembled large islet cells, they failed to take any of the special stains for alpha and beta granules. None showed mitoses. In some areas strands of tumor cells were separated only by capillaries; in other areas by wide bands of hyalinized collagen. Stages of transition between these two extremes showed that collagen was laid down in increasing amounts along the walls of capillaries and later became hyalinized and relatively acellular. This hyaline material, similar to that often seen in islet cell adenomas, did not take stains for amyloid. Some hyalinized centers contained diffuse deposits of calcium. There was no relationship of the tumor nodules to the lobular structure of the liver. The general architecture and cell types were similar to those of the primary tumor. Degenerative changes were common. Clear vacuoles, relatively small and uniform in size, appeared in the cytoplasm. Some cells were packed with these vacuoles, outlined by traces of basophilic substance, and had centrally placed nuclei. Other cells were almost colorless. Foci of coagulation necrosis of tumor cells were present. Assays of insulin on the primary tumor and on the metastatic lesions in the liver were attempted, but extraction was not successful. In the surrounding liver tissue there was a striking fatty metamorphosis. While some liver cells contained a single vacuole which displaced the nucleus to one side, the predominant picture was one of cells filled with fine coalescing vacuoles in a colorless cytoplasm, leaving the nucleus centrally placed. Best's carmine stain after absolute alcohol fixation showed a moderate amount of glycogen.

The residual *pancreas* weighed 32 gm. and showed only a few minute foci of fat necrosis in the peripancreatic fat immediately adjacent to the site of operation. The pancreatic duct was patent. There was no evidence of residual tumor. The tail of the pancreas beyond the operative site showed no gross lesions. Microscopically, there were no significant changes. In the portion from the tail of the pancreas a pancreatic duct of moderate size was surrounded by large groups of islet cells, but no acini were evident. There was good differentiation between alpha and beta cells, and the latter tended to be finely vacuolated. In the remainder of the pancreas, the beta cells had few granules, but there was no evidence of hydropic degeneration or hyalinization of any islets.

The *pituitary gland* weighed 700 mg. and grossly was not remarkable. When semiserial sections were cut and stained with hematoxylin

and eosin, the central half of the anterior lobe was seen to be composed of massed acidophilic cells surrounded by a narrow border of mixed eosinophilic, basophilic and chromophobe cells. The peripheral half showed a marked predominance of basophilic cells, a scattering of eosinophilic cells and a reduction in chromophobe cells. The basophilic cells tended towards an adenomatous arrangement. The majority of these cells contained deeply stained granules; others, light blue-staining granules. Vacuoles in the cytoplasm were prominent and numerous. Throughout the lobe were many small spaces surrounded by mixed cells and filled with colloid material which was usually acidophilic. A few, however, were lined entirely by basophilic cells and filled with basophilic colloid. The pars intermedia was marked by large, elongated acini lined by flat, slightly basophilic epithelial cells and filled with abundant acidophilic colloid. The posterior lobe was structurally normal, but heavily infiltrated anteriorly by basophilic cells.

Comment

Clinical Observations

The eight patients previously reported (Table I), as well as the patient herein reported, died, most of them within 1 year of first symptoms whether or not operation had been done. In case no. 9, the long duration, over 4 years, is interesting. The progressive nature of the symptoms was characteristic in the histories of all nine patients in that hypoglycemic attacks eventually occurred and were finally difficult to control even when a constant feeding regime was carried out.

The obesity in case no. 9 was extreme. Weight gain was mentioned in only three of the cases (nos. 1, 2 and 8) though it has been commonly stated in reports of islet cell adenomas.

The development of transitory hyperglycemia and glycosuria, indistinguishable from diabetes mellitus, in the postoperative period occurred only in case no. 9. It is interesting also that this is the single instance where the primary tumor was completely removed. One could speculate that the metastatic lesions were non-functioning, but such speculation would necessitate the acceptance of the theory that the pancreas also was not producing sufficient insulin to maintain the blood sugar within normal limits and the urine sugar-free. It should be noted that the hyperglycemia and glycosuria occurred after the administration of intravenous glucose had been stopped. Furthermore, the patient had normal blood-sugar determinations and no symptoms of hypoglycemic shock during the 10-day period prior to death, and no insulin was given. The picture was further complicated by infection. Hence no conclusions are drawn.

Summary of Nine Cases of Islet Cell Tumors with Metastases

Case no.	Author	Age	Weight increase (pounds)	Total length of symptoms (months)	Operation	Result	Assay of insulin	Autopsy
1	Wilder, Allan, Power and Robertson[2]	40	25	21	Exploratory: inoperable carcinoma	Died postoperatively	Present in liver nodule	Metastases in liver, lymph nodes and mesentery; pituitary not mentioned
2	Judd, Faust and Dixon[3]	18	30	4.5	Exploratory: tumor of pancreas and multiple metastases in liver; biopsy of liver nodule	Died postoperatively	Not done	Not done
3	Bickel, Mozer and Junet[4]	56	Not mentioned	7	None	Died without operation	Present in primary growth; liver nodule negative	Metastases in liver, peritoneum and epicardium; interstitial pancreatitis; pituitary not mentioned
4	Cragg, Power and Lindem[5]	51	Not mentioned	9	Exploratory: biopsy of liver	Died postoperatively	Present in liver nodule	Metastases in liver and lymph nodes; diffuse tumor in pancreas; pituitary not examined
5	Joachim and Banowitch[6]	31	Not mentioned; tendency towards obesity	2	Resection of spleen and portion of tumor in tail of pancreas and a lymph node showing metastases; liver apparently negative	Died postoperatively	Not done	Not done
6	Flinn, Beatty, Ginsberg and Hemsath[8]	45	Not mentioned	5	None	Died without operation	Not carried out successfully	Tumor in head of pancreas; metastases in liver and lymph nodes; pituitary "grossly" normal
7	Jacobsen[9]	36	Not mentioned	12–16	Exploratory: inoperable carcinoma in pancreas; metastases in liver	Died postoperatively	Not done	Tumor in pancreas eroded into duodenum; metastases in liver; pituitary not mentioned
8	Cannon (personal communication) See also references 10 and 11	36	16	12	Exploratory: inoperable carcinoma in pancreas; metastases in liver	Died postoperatively	Inconclusive	Metastases in liver, lymph nodes, adrenals, spine, lungs and pleura; pituitary not examined
9	Gray	48	65	48	Resection of pancreatic tumor; biopsy of metastatic lesion in liver	Died postoperatively	Not carried out satisfactorily	Metastases in liver; marked basophilism of pituitary gland

Pathological Observations

The cells of both the primary and the metastatic lesions bore a striking histological resemblance to islet cells. The fact that beta cells were demonstrated in the pancreatic tumor, but not in the metastatic lesions, is interesting, but in the absence of satisfactory assays of insulin, no definite correlation with the clinical picture may be drawn. Indeed, it has been stressed by Campbell, Graham, and Robinson [14] that, when the amount of tumor is known and when careful assays of insulin have been carried out and specific islet cell types identified, the information may not be closely correlated with the clinical picture.

In eight cases metastases were localized in the liver, mesenteric lymph nodes and epicardium. In case no. 8, however, generalized metastases were found and the widespread nature of the metastases leaves no doubt that they were carried in the blood stream.

The basophilism of the pituitary gland in case no. 9 is striking. The massed eosinophils, while prominent, are probably not significant. No histological changes in the pituitary glands were recorded in the other eight cases. Increase in basophilic cells has been described in cases of islet cell adenoma with hypoglycemia. Friedman's [15] two cases showed marked basophilic adenomatous hyperplasia of the anterior lobe and marked obesity. He reviewed all the other cases to date in which pituitary lesions associated with islet cell tumors were described. Many observers [15–17] had noted in those suffering from obesity larger numbers of basophils as well as more basophilic and transitional cell adenomas than in non-obese persons.

Summary

A case of functioning islet cell carcinoma of the pancreas with metastases in the liver is reported and compared with eight other proved islet cell carcinomas with metastases. The development of a transient hyperglycemia and glycosuria in the postoperative period following removal of the primary tumor is noted. Beta cells were demonstrated in the primary tumor, but not in the metastatic lesions. Pituitary basophilism also was found.

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DESCRIPTION OF PLATE

PLATE 100

FIG. 1. General topography of primary tumor showing invasion of capsule. × 17.

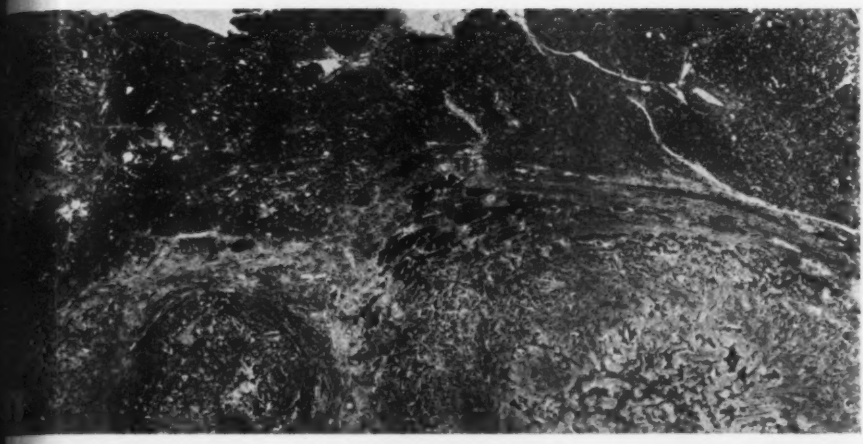
FIG. 2. Primary tumor. The cells are arranged in islet-like strands. × 250.

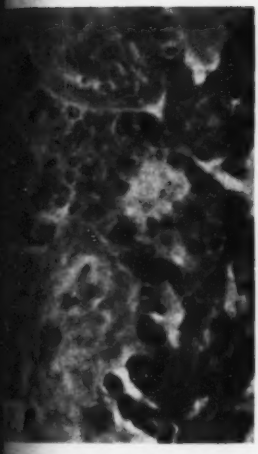
FIG. 3. Primary tumor. The cells tend to form ductlike structures. × 250.

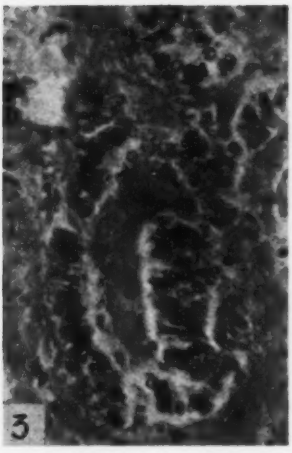
FIG. 4. Primary tumor. The cells are grouped in alveolar-like masses. × 250.

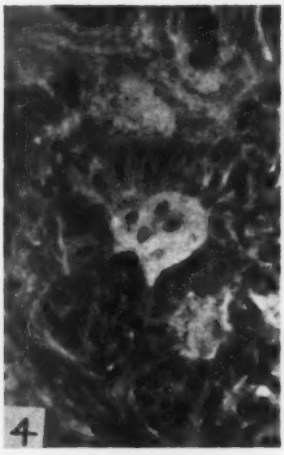
FIG. 5. Tumor in liver. Marked hyalinization. The surrounding liver tissue shows fatty metamorphosis. × 40.

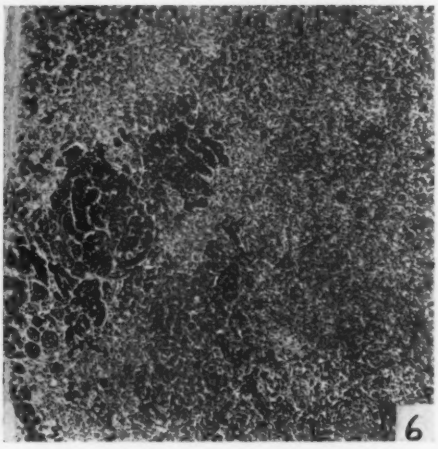
FIG. 6. Pituitary gland. Deep-staining basophilic cells arranged in adenomatous masses. ×23.











Functioning Islet Cell Carcinoma

GLYCOGEN INFILTRATION OF THE LIVER CELL NUCLEI *

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One of the most important functions of the liver is the synthesis of glycogen, a storage form of carbohydrate. This is a labile substance readily convertible into glucose, and although it is present in abundance in the cytoplasm of the liver cells after the ingestion of carbohydrates, it decreases or even disappears during fasting or starvation. On the other hand, glycogen is not found normally in the nuclei of the liver cells at any time. This report is devoted chiefly to a study of the pathological infiltration of liver-cell nuclei by glycogen, but during the course of this study it was possible to confirm certain earlier observations regarding the normal storage of glycogen in liver cells. These observations will first be reviewed briefly because the facts established by histological studies of glycogen deposition in the liver seem not to be very widely known.

Fixation and Staining

Glycogen is the only carbohydrate that can be studied histologically, being stained a brownish red colour by iodine and crimson by the more widely used Best's carmine stain. It is water-soluble and in order to retain it in the tissue for examination, water and predominantly aqueous solutions must be avoided before and after fixation. Ordinarily this end is achieved by fixing blocks of tissue in absolute alcohol and embedding them in celloidin.[1] However, in this study the following technic was employed:

Thin blocks of liver were fixed in a solution made up of 9 parts of absolute alcohol and 1 part of 40 per cent formaldehyde.† After fixation for 24 hours or longer the blocks of tissue were dehydrated in the usual manner, beginning with 95 per cent alcohol, and embedded in paraffin. Sections were cut at a thickness of 6 μ and mounted on ordinary glass slides. The paraffin was removed by xylol and the sections were then coated with a thin film of celloidin by pouring on a few drops of a 0.3 per cent solution of celloidin in equal parts of absolute alcohol and ether. Then the sections were run down to water following which the ordinary Best's carmine method of staining[1] was applied, the sections were freed from celloidin by acetone, dehydrated and mounted in Canada balsam.

By this method, thin sections can be readily obtained and handling of the sections is simplified by the fact that they are mounted on glass

* Received for publication, September 22, 1941.

† Dr. Shields Warren, in a personal communication, stated that he has found Carnoy's fluid a more satisfactory fixative than either absolute alcohol or the formaldehyde and absolute alcohol solution used in this study.

slides at the outset. Altogether the procedure outlined is much quicker and easier than the usual celloidin method and gives equally reliable results.

Intracytoplasmic Glycogen

Best's carmine stain reveals the glycogen, if present, as small or large crimson granules distributed through the cytoplasm of the liver cells. Tissue culture studies,[2] however, have shown the glycogen to be actually in solution in the cytoplasm of the living cells and therefore its granular appearance in stained fixed tissue must be regarded as a constant fixation artefact. In livers containing large amounts of glycogen it is found to be fairly evenly distributed throughout the liver lobule, but as it is released from the liver it disappears first from the central cells of the lobule. Consequently, when present at all it is generally found to be most abundant in the periportal portions of the lobule. A tendency for the glycogen granules to be concentrated in the same side of each cell is due to the alcohol fixative having driven the glycogen ahead of it as it penetrated the tissues.[1]

In a large series of autopsy cases, in which the livers were stained for glycogen in the present study, a correlation could generally be shown between the amount of glycogen present and the elapsed time since food intake before death. In cases where death is unexpected and sudden, as from physical trauma or pulmonary embolism, glycogen is generally abundant in the liver. On the other hand, when there is a long agonal period of starvation, glycogen is generally scanty or absent.

The interval between death and the time of fixation of the liver up to 8 or 10 hours does not materially affect the appearance and quantity of glycogen in the liver, as estimated in sections stained with Best's carmine. Once the tissue is fixed in the alcohol-formaldehyde solution the glycogen apparently will remain unchanged for indefinite periods. Even after several years formaldehyde-fixed tissues will sometimes show the glycogen granules fairly well.

It is generally known [3] that the presence of large amounts of glycogen in the liver-cell cytoplasm will cause a characteristic appearance in ordinary paraffin sections stained with haematoxylin and eosin (Fig. 1). The cytoplasm, instead of presenting its more usual homogeneous character, becomes pale and fluffy in appearance. This appearance is sometimes mistaken for a degenerative change and it should be emphasized that it merely denotes the normal presence of glycogen which, of course, actually has been dissolved out during the fixation and staining process. Although this fluffy appearance is characteristic

and constant when large amounts of glycogen are present, lesser amounts of glycogen may cause scarcely any discernible change in the appearance of the cytoplasm in paraffin sections. Consequently, in order to exclude or detect the presence of small quantities of glycogen in the cytoplasm of liver cells, resort must be had to the Best's carmine stain. Thus the liver-cell cytoplasm may or may not contain glycogen under normal conditions.

Intranuclear Glycogen

The liver-cell nuclei normally do not contain glycogen. However, in 1883 Ehrlich [4] noted large glycogen-filled vacuoles in the liver cells in cases of diabetes mellitus. Later these vacuoles were shown to be the nuclei and since then it has been generally recognized that glycogen often infiltrates the liver-cell nuclei in diabetic persons. It has also been known for a long time, though not widely recognized, that glycogen frequently can be demonstrated in the liver-cell nuclei in non-diabetic persons.[5] For this reason Warren [5] believes that the mere presence of glycogen in the nuclei should not be regarded as a particularly significant lesion in diabetes. He does emphasize, however, that there is a significant reciprocal relation between the amounts of intranuclear and of intracytoplasmic glycogen. He also presents evidence to show that insulin promotes the deposit of glycogen in the liver-cell cytoplasm. In other words, he suggests that in the diabetic patient there tends to be an absence of glycogen-containing nuclei in the liver if he is well treated with insulin, while in uncontrolled cases glycogen-containing liver-cell nuclei are likely to be present in abundance.

In ordinary paraffin sections of the liver from cases of uncontrolled diabetes, one frequently sees peculiar vacuolated, empty-appearing nuclei in large numbers, situated in the peripheral cells of the liver lobules. It is usually assumed that these nuclei are the glycogen-containing ones. Similar vacuolated nuclei in similar distribution are also frequently seen in the liver in non-diabetic cases. In our earlier autopsy records this vacuolation of liver-cell nuclei has often been described as though representing some form of degenerative change, but almost never has there been any speculation as to the nature of the abnormality. It was the purpose of the study here reported to determine whether these vacuolated nuclei in the liver cells of diabetic patients are actually the ones which contain glycogen; whether this peculiar vacuolation of liver-cell nuclei is always associated with the intranuclear accumulation of glycogen and whether the presence of any considerable quantity of glycogen in the nucleus always produces

vacuolation visible in paraffin sections. In short, can this type of vacuolation of liver-cell nuclei be depended upon as a reliable indication of the presence of intranuclear glycogen?

The appearance, if not the nature, of these peculiar vacuolated liver-cell nuclei as seen in paraffin sections stained with haematoxylin and eosin is probably familiar to all who see routine autopsy material (Figs. 2 and 3). They are nearly always confined to the liver cells at the periphery of the lobules about the portal areas, although when present in large numbers they may approach the central part of the lobules. Thus, they occur predominantly in the cells which, during depletion of liver glycogen, are the last to give up their cytoplasmic stores of this carbohydrate. In their most striking form these vacuolated nuclei are very large, measuring up to 25 μ or more in diameter; they are generally somewhat irregular in outline, even crescentic or crenated. The nuclear chromatin and the nucleolus, if it is visible, are pushed to the periphery of the nucleus so that the nuclear membrane appears to have been overtraced with deep purple dye. The central part of the nucleus either appears completely empty and unstained, or it presents a pale, slightly opaque, violet or gray homogeneous appearance. The empty-appearing nuclei are indeed just that, for the diameter of the swollen nuclei being greater than the thickness of the histological section, the tops and bottoms of these nuclei have been cut off and one is in reality looking through an empty hole in the section. If histological sections of increasing thickness are prepared, the holes finally disappear in the thicker sections and only the nuclei with gray homogeneous centres remain. The latter, therefore, represent empty nuclei in which the nuclear membrane is intact over the upper or lower surface. By focusing up or down, this part of the nuclear wall is often clearly revealed.

In order to study the relation of this characteristic vacuolation of liver-cell nuclei to the intranuclear accumulation of glycogen, blocks of liver from 160 consecutive autopsy cases were fixed and stained for glycogen by the modification of the Best technic already described. Only newborn infants were excluded from this series, since preliminary studies had revealed no instance of vacuolation of the liver-cell nuclei in the newborn. The routine paraffin sections of the livers from the same 160 cases were examined, as well as the special sections stained for glycogen, but the examination of the two types of preparation was carried out independently and in each instance the specimen was graded as 0, 1, 2, 3 or 4 according to the examiner's estimate of the abundance of the peculiar vacuolated nuclei, described above, in the sections stained with haematoxylin and eosin, or the abundance of glycogen-containing nuclei in the sections stained with Best's carmine.

Without exception it was found that in those cases in which the peculiar vacuolated liver-cell nuclei were present in the routine sections, a roughly equivalent number of nuclei could be shown to contain glycogen in the liver sections stained with Best's carmine. The closeness of the numerical correspondence between the abnormal nuclei in the two types of preparation is indicated by the fact that in no instance was there a major disagreement between the grades which had been assigned to represent the estimated numbers of abnormal nuclei. Actually, the nuclei in question were more readily detected in sections stained with haematoxylin and eosin than in those stained for glycogen. That the empty-appearing nuclei seen in the former type of preparation really corresponded with those which contained glycogen in the sections stained with Best's carmine could scarcely be doubted after comparison of the sections under the microscope. Apart from the presence of one or more droplets of crimson-stained intranuclear glycogen in the sections stained to demonstrate its presence, the morphology of the abnormal nuclei was identical in the two types of histological preparation (Figs. 3 and 4). There was no instance of glycogen infiltration of liver-cell nuclei in which the peculiar nuclear vacuolation was lacking. Among the 160 cases studied, 94 were completely negative, that is, the liver-cell nuclei neither contained glycogen nor showed any sort of vacuolation. In 66 cases, or 41 per cent, small or large numbers of liver-cell nuclei were vacuolated and contained glycogen; in 24 of these cases, or 15 per cent of the total, considerable numbers of nuclei were found to be vacuolated by the presence of glycogen, and the abnormal nuclei were present in sufficient abundance that the sections had been assigned grades of 3 or 4.

The positive and negative evidence accumulated by the study of this first group of cases was supplemented by further evidence on the positive side obtained from the study of an additional group of 155 autopsy cases which were dealt with in the following manner. Blocks of liver tissue from each case were preserved in the alcohol-formaldehyde fixative in addition to the blocks of tissue taken routinely. The routine paraffin sections of liver from these same cases were studied and graded, as before, according to the numbers of abnormal vacuolated nuclei. In each case in which the peculiar vacuolated liver-cell nuclei were found in the routine sections, further histological sections were prepared from the specially fixed liver tissue and stained for glycogen. Again, in every instance, the peculiar empty-appearing nuclei seen in the routine liver sections stained with haematoxylin and eosin proved, in the sections stained for glycogen, to be glycogen-containing. In this group of 155 cases, 57 cases, or 37 per cent, showed the pres-

ence of vacuolated glycogen-containing liver-cell nuclei, and in 20 cases, or 13 per cent of the total, these abnormal nuclei were present in large numbers. This group of cases, combined with the first group studied, makes up a total of 315 cases. Vacuolated glycogen-containing liver-cell nuclei were demonstrated in 123 of these cases, or in 39 per cent, while in 44 cases, or in 14 per cent of the total, the abnormal nuclei were present in large numbers.

These findings, showing as they do a perfect correlation between glycogen infiltration and vacuolation of the liver-cell nuclei, were deemed sufficient to justify the conclusion that glycogen infiltration of the nuclei of the liver cells regularly produces a peculiar vacuolation of these nuclei which is easily recognizable in paraffin sections stained by routine methods. Moreover, these results indicate that this peculiar vacuolation does not occur except in association with infiltration of the nucleus by glycogen. It can therefore be stated that the characteristic vacuolation of liver-cell nuclei, already described, can be depended upon as a reliable indication of the presence of intranuclear glycogen.

Analysis of Cases of Intranuclear Glycogen Deposit

In the hope of determining the causes of glycogen infiltration of the liver-cell nuclei, the clinical and autopsy records were studied carefully in a series of cases in which large numbers of glycogen-containing nuclei were present in the liver. Only 44 such cases had been found among the 315 cases, as has been described in the preceding section of this report. However, the conclusions drawn from that study were made use of in the selection of additional cases of marked glycogen infiltration of the liver-cell nuclei. The routine sections, stained by haematoxylin and eosin, of the livers from 932 consecutive cases from the autopsy files, were examined with a view to selecting those cases in which the presence of many characteristically vacuolated liver-cell nuclei gave clear indication of glycogen infiltration of a degree equivalent to that found in the original 44 cases. This search yielded 96 additional cases, bringing the total to 140 cases available for analysis.

The hospital records of these 140 cases were examined. Among the features, other than the clinical diagnosis, of which particular note was taken were such factors as age, sex, total duration of illness, duration of severe illness, duration of critical illness, interval between the last intake of food and death, the use and quantity of intravenous glucose during the final illness, history of diabetes mellitus, the urinary findings, blood chemistry, blood pressures, blood counts, presence of fever, duration of fever, medication and symptoms such as dyspnoea and cyanosis. The autopsy protocols of these cases were likewise reviewed

and note taken of the principal anatomical findings, as well as other factors of possible importance such as state of nutrition and degree of arteriosclerosis. The histological sections of the liver in each case were reëxamined for lesions other than glycogen infiltration of the nuclei.

Clinical Features

It became apparent almost from the beginning of this analysis that many different kinds of cases were represented. Of the 140 cases, 32, or 23 per cent, were cases of diabetes mellitus. Since it is well established that glycogen infiltration of the liver-cell nuclei is common in diabetes, it was important to exclude the possibility of diabetes in the remaining supposedly non-diabetic cases. In deciding whether or not any given case was one of diabetes, the chief determining factor had to be whether or not such a diagnosis had been made clinically, but cases were considered as being cases of diabetes if there was any hint at all that they might have been. The figure of 23 per cent is therefore a maximum figure. On the other hand, in the cases considered to be non-diabetic, urinalysis showed the absence of sugar; blood sugar, when determined, was within normal limits, and nowhere in the case histories was the possibility of diabetes entertained. Perhaps if all the facts had been known, some of these cases might have proved to be cases of diabetes, but undoubtedly the great majority of the 108 cases considered to be non-diabetic were truly non-diabetic.

In only one case of diabetes was death attributed to uncomplicated diabetic coma, and for further consideration of the cases, diabetes, *per se,* was regarded simply as one of the many possible factors which might be responsible for glycogen infiltration of the liver-cell nuclei. The diabetic and non-diabetic cases were, therefore, considered together in the analysis of the cases for other factors of possible importance. This study did not reveal any other factor of constant causal significance nor any factor which was common to all cases, but the following features, briefly recorded, seemed noteworthy.

Among the 140 cases analyzed, the ages varied from 11 months to 82 years with an average of 53 years. It was apparent that the age of the patients was related to the disease from which they died and that there was no correlation between age and the presence or absence of glycogen in the liver-cell nuclei. Males constituted 54 per cent of the cases and females 46 per cent, a proportion between the sexes which does not differ greatly from that encountered within recent years among the cases coming to autopsy in this department.

No correlation could be established between the duration of illness and the presence of glycogen-containing nuclei in the liver. The short-

est interval between the onset of disease and death in a person previously in apparently good health was in a child, 2 years of age, who had died of diphtheria on the sixth day of illness. No correlation was observed with food intake nor with the intravenous administration of glucose. Likewise the medication employed in different cases presented no features common to all cases.

The blood pressure was moderately or extremely elevated in 40 per cent of the cases. There were no consistent findings on examination of the blood. However, the white blood cell count was elevated in many instances.

Associated Anatomical Lesions

The anatomical diagnoses recorded in the 140 cases covered a wide range, but it is noteworthy that there did not occur in the series a single case of uncomplicated traumatic death. This merely serves to emphasize the fact that glycogen infiltration of the liver-cell nuclei does not occur under normal conditions. The body was described as of average nutrition in 55 per cent of the cases, as obese in 25 per cent and as cachectic in 20 per cent.

Because of the variety of lesions, the cases could be grouped only in the most arbitrary manner according to the nature of the principal lesions which recurred most frequently. There was sometimes overlapping of certain features among cases placed in separate groups.

In 44 cases, or 31 per cent of the total, the outstanding feature at autopsy was the presence of marked inflammatory lesions usually accompanied by more or less extensive necrosis of tissue. Typical of such lesions were instances of multiple pulmonary abscesses, bilateral pyonephrosis, actinomycosis, gangrene of the lower extremities with septicaemia, phlegmon of the abdominal wall and extensive necrosis and secondary infection of bladder and vagina associated with carcinoma of the cervix. The locations of such lesions were most varied. Thirteen of the 32 cases of diabetes mellitus fell into this group.

In 70 per cent of the cases there was moderate or marked arteriosclerosis. There were 31 cases in which the principal diagnosis was some cardiac lesion leading to heart failure. In 14 of these cases there was coronary thrombosis and myocardial infarction. In 19 cases the principal lesion was a cerebrovascular one, either cerebral softening or haemorrhage. In combining these two latter groups, totaling 50 cases, or 36 per cent of the whole series, it is interesting to note that there were 5 cases in which there was a necrotizing arteriolosclerosis. Among these cases, too, necrosis of tissue was present in 33 instances in the form of myocardial infarction or encephalomalacia. Fourteen of the 32 cases of diabetes fell into this combined group of 50 cases.

There were 25 cases in which the outstanding lesion was a neoplasm. Among these were carcinomata of the breast, tongue, thyroid, larynx, bronchus, stomach, gallbladder, rectum and cervix. There was one lymphosarcoma, a chromaffin tumour of the adrenal, 2 cases of chromophobe adenoma of the pituitary, a suprasellar craniopharyngioma, a colloid cyst of the third ventricle and a glioblastoma. In many instances, extensive tumour metastases and necrosis with secondary infection were associated findings.

There were three cases of tuberculous meningitis, in two of which there was also a generalized miliary tuberculosis. All of these showed clinically a marked increase in intracranial pressure, and large tuberculomata of the brain were demonstrated in each case at autopsy.

One small but interesting group was made up of 4 cases of diphtheria in young children. In each, the diagnosis of diphtheria was established both clinically and pathologically. It is interesting to note that in 1900, Councilman, Mallory and Pearce,[6] in an extensive study of the pathology of diphtheria, described a peculiar vacuolation of the liver-cell nuclei as a frequent but inconstant finding. They regarded the lesion as a rare form of degeneration of the affected nuclei, but they realized that it was not peculiar to diphtheria for they noted that many such nuclei had been observed in a case of leukemia. Examination of the sections of liver from other cases of diphtheria in the autopsy files of this department revealed glycogen-containing nuclei in large numbers in at least one-half of the cases, but they were not a constant finding.

The remaining 14 cases could not easily be grouped or else were single examples of disease entities. There was 1 case of myasthenia gravis and 1 in which the patient died of pulmonary embolism 14 days after pregnancy had been terminated because of extreme toxemia.

A regrouping of some of the cases was prompted by consideration of the four cases of diphtheria, all of which were brought to hospital late in the illness and showed clinically symptoms and signs of suffocation. In six other cases as well, cyanosis and signs of strangulation were outstanding clinical features. One of these was a case of carcinoma of the tongue in which death finally occurred from respiratory obstruction. The same mode of death was observed in a case of carcinoma of the larynx, in another in which a large collar-like carcinoma of the thyroid compressed the trachea, and again in a case of extensive tumour infiltration of the mediastinum from a primary carcinoma of the breast. Another similar case was that of a patient with uncontrollable asthma who finally died with extreme respiratory embarrassment. The sixth case of this type was one in which the patient, a man 69 years old, dying of pneumonia, was found at autopsy to have an extremely nar-

row, sabre-shaped, calcified trachea. Dyspnoea and cyanosis were prominent in many of the cardiovascular cases also and in other cases with extensive pulmonary lesions such as abscesses, bronchial carcinoma or tuberculous cavitation. These could possibly be placed in the same group, but in them respiratory embarrassment was not so extreme nor could it be so readily evaluated from anatomical findings.

The four cases of tumour centering around the pituitary or floor of the third ventricle, as well as the one case of cerebral glioma and the three cases of tuberculous meningitis with markedly increased intracranial pressures, were thought possibly to form a significant group. However, examination of the histological sections of the liver in additional selected cases of this kind, in search of glycogen-containing nuclei, failed to reveal any constant correlation.

Apart from glycogen infiltration of the liver-cell nuclei, the liver itself in many cases showed no pathological alterations and in the remaining cases all manner of changes were observed. Chronic passive congestion of varying degree was a frequent occurrence, while other findings included fatty metamorphosis, periportal hepatitis and secondary carcinoma. Cirrhosis of the liver was present in only one case.

Thus, the analysis of 140 cases of marked glycogen infiltration of the liver-cell nuclei demonstrated only that this abnormality occurs as an accompaniment of a considerable variety of diseases. The more frequently associated conditions were diabetes mellitus; acute suppurative inflammatory processes; neoplasms; inflammatory, arteriosclerotic and neoplastic lesions of various kinds in which necrosis of tissue was a prominent feature, and lesions causing respiratory embarrassment with relative anoxia. The analysis provided no real clue as to the ultimate cause of glycogen infiltration of the liver-cell nuclei.

The grouping of the abnormal glycogen-containing nuclei in the periportal regions of the liver lobules suggests the possibility that some factor referable to the blood supply may be responsible for the occurrence of glycogen infiltration of the nuclei in these areas rather than elsewhere in the liver lobules. A significant relationship is also suggested by the fact that the periportal liver cells in which the abnormal nuclear accumulation of glycogen occurs are those which normally, during depletion of liver glycogen, are the last to give up their cytoplasmic glycogen stores.

Summary and Conclusions

Peculiar empty-appearing or vacuolated liver-cell nuclei with a peripheral arrangement of the chromatin are described as occurring not infrequently in paraffin sections of the liver from routine autopsy cases when stained by haematoxylin and eosin. By means of parallel glyco-

gen staining of sections of the liver, it is demonstrated that such abnormal, vacuolated nuclei invariably contain glycogen. Conversely, it is shown that glycogen infiltration of the liver-cell nuclei regularly produces the characteristic vacuolation of these nuclei which is easily recognizable in paraffin sections stained by routine methods. Such vacuolation of the liver-cell nuclei can, therefore, be depended upon as a reliable indication of the presence of intranuclear glycogen.

In a series of 315 unselected autopsy cases from which only newborn infants were excluded, glycogen infiltration of the liver-cell nuclei was demonstrated by glycogen staining in 123 cases, or in 39 per cent of the total. The abnormal glycogen-containing nuclei were present in large numbers in 44 cases, or in 14 per cent of the whole series.

In a series of 140 cases in which large numbers of liver-cell nuclei contained glycogen, the clinical data and autopsy findings were analyzed in the hope of determining the causes of glycogen infiltration of the liver-cell nuclei. Cases of diabetes mellitus made up 23 per cent of the series. The other cases, as well as the diabetic cases, showed a great variety of lesions. The analysis of clinical and autopsy data failed to reveal any factor of constant causal significance other than the presence of uncontrolled diabetes mellitus. Nevertheless, many of the features of the cases studied seemed worthy of note and these have been briefly recorded. The ultimate cause of glycogen infiltration of the liver-cell nuclei remains unknown.

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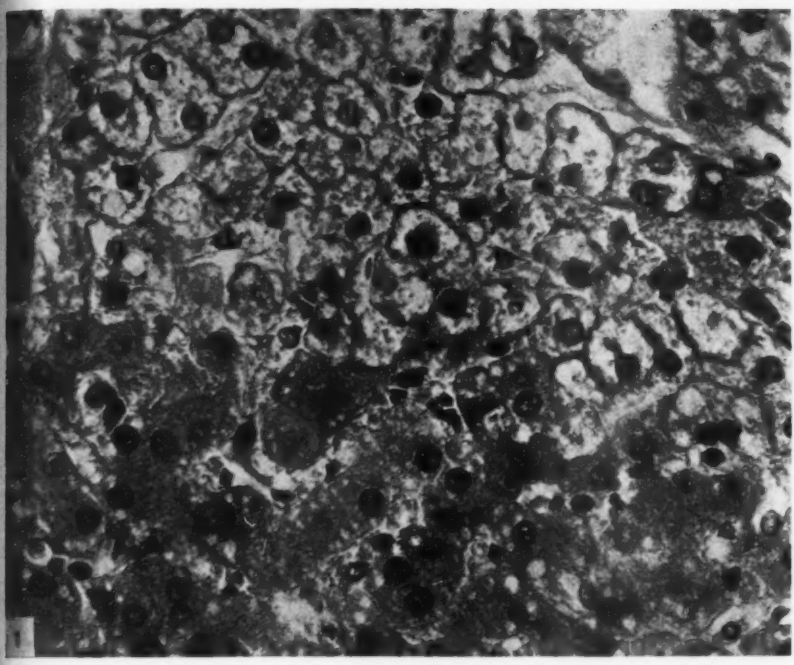
DESCRIPTION OF PLATES

PLATE 101

FIG. 1. Paraffin section of liver stained with haematoxylin and eosin, showing a patch of liver cells above in which the pale, fluffy cytoplasm is indicative of a rich glycogen content. These contrast with glycogen-poor cells below in which the cytoplasm has the more usual uniformly granular appearance. × 565.

FIG. 2. Paraffin section of liver stained with haematoxylin and eosin, showing large numbers of vacuolated, empty-appearing nuclei, particularly abundant near the periphery of the lobule. × 350.



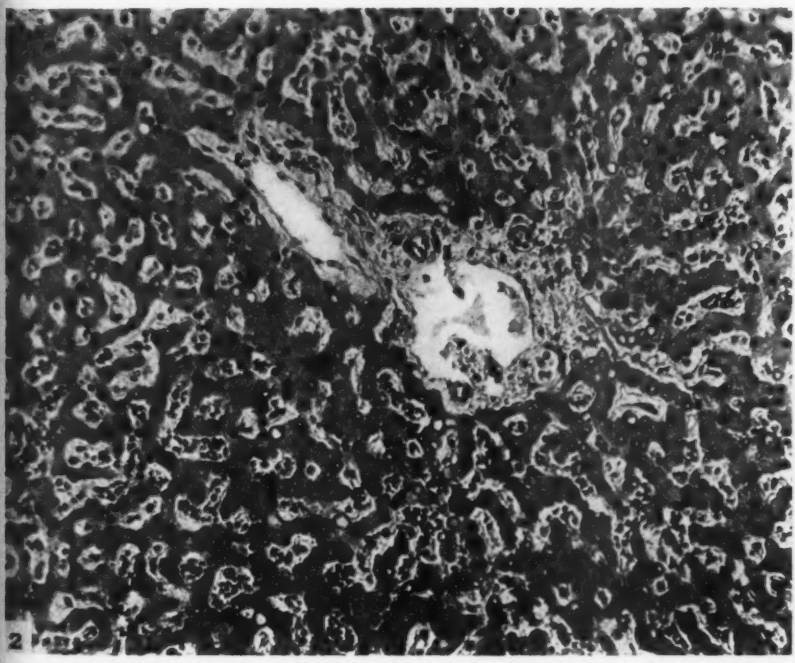
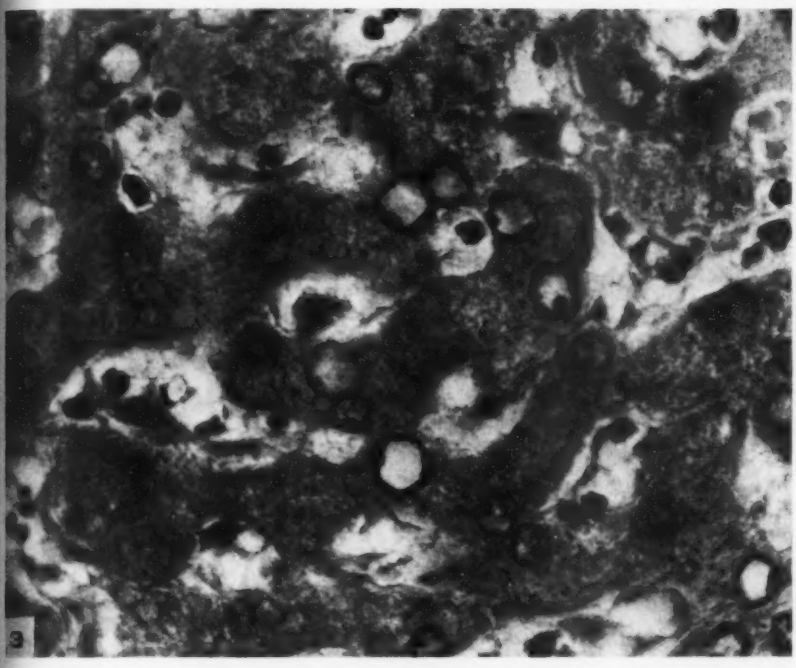
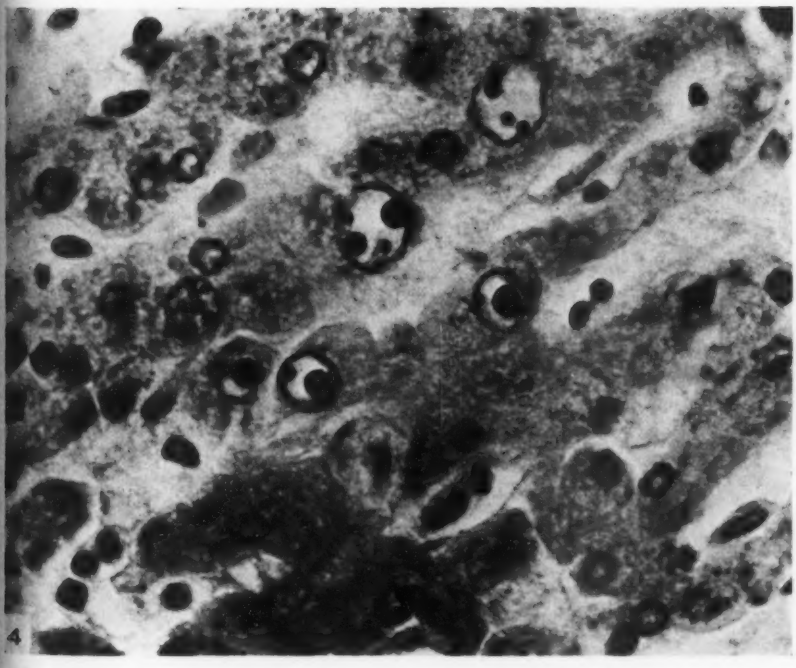


PLATE 102

FIG. 3. Paraffin section of liver stained with haematoxylin and eosin, showing the characteristic vacuolated liver-cell nuclei. Chromatin and nucleoli occupy an extreme peripheral position. × 855.

FIG. 4. Celloidin-treated, paraffin section of liver stained by Best's carmine method, showing glycogen infiltration of six of the liver-cell nuclei. The crimson-stained glycogen is represented in the photomicrograph by the black intranuclear masses. Apart from the presence of these droplets of glycogen, the appearance of these nuclei is identical with that of the vacuolated nuclei shown in Figure 3. × 855.





Hoge and Duff Glycogen Infiltration of Liver Cell Nuclei

EFFECTS OF YEAST AND FOOD INTAKE ON EXPERIMENTAL CARBON TETRACHLORIDE CIRRHOSIS OF THE LIVER IN THE RAT *

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Clinical studies on cirrhosis of the human liver have indicated that beneficial effects are produced by feeding a highly nutritious diet supplemented with vitamin B concentrates.[1,2] Nakahara, Mori and Fujiwara [3] have stated that the addition of baker's yeast to the diet protects the rat against the development of hepatic cirrhosis and hepatomas produced by p-dimethyl-amino-azo-benzol. This has been confirmed with brewer's yeast by Sugiura and Rhoads.[4] Von Glahn and Flinn [5] have reported that feeding brewer's yeast partially protects rabbits against cirrhosis produced by feeding lead arsenate. That liver damage may be produced by a diet deficient in factors contained in yeast has been indicated by György and Goldblatt [6] for the rat and by Rich and Hamilton [7] for the rabbit. Recently other reports suggest that other food components may be involved in the production of experimental cirrhosis.[8]

The present report describes the effects of the administration of brewer's yeast † on CCl_4 cirrhosis in the rat. Both developmental and recovery stages of the disease were studied.

It is well known that yeast stimulates the appetite (Cowgill,[9] Drill and Sherwood [10]). In the present studies, therefore, the effect of increased food consumption due to the appetite-stimulating action of yeast was controlled. This was done by feeding the same amount of food to the animals while varying the quantities of yeast in the diet. In this manner the essential variable of the experiment was the quantity of yeast consumed.

PART I. EFFECT OF FEEDING BREWER'S YEAST ON THE DEVELOPMENT OF CIRRHOSIS OF THE LIVER INDUCED BY CCl_4 IN THE RAT

Two sets of experiments were performed. The first, series I, was a comparison of the effects of 1.5 per cent to 1.0 per cent with those of 8 per cent to 10 per cent brewer's yeast supplements. The former concentration was found to be adequate for good growth in normal animals. The second set of experiments, series II, was a comparison of the effects

* Received for publication, November 3, 1941.

† Harris' medicinal brewer's yeast powder, Tuckahoe, N.Y.

of 0.75 per cent to 0.2 per cent with those of 10 per cent brewer's yeast supplements. The lower concentration was inadequate for good growth in normal animals.

SERIES I

Methods

Male rats of Sherman stock, 10 weeks old, were employed. They were injected subcutaneously with a 50 per cent solution of CCl_4 in mineral oil, twice weekly, in a dosage of 0.05 cc. per 100 gm. of body weight. The basal diet employed was: sucrose, 67 per cent; casein, 20 per cent;* butter, 8 per cent; Hawk-Oser [11] salt mixture, 4 per cent. One group of animals received this basal diet to which was added brewer's yeast in amounts of 1.5 per cent decreasing to 1.0 per cent. Another group of animals received the same basal diet except that the casein was 18 per cent; the sucrose, 62 to 60 per cent, and the brewer's yeast, 8.0 to 10 per cent. The decrease in the casein and sucrose concentration corrected for the additional protein and caloric values of the large yeast supplements.[12] To determine the influence of the diets on growth, two groups of rats received the same diets without being treated with CCl_4. This also offered an opportunity to study the effects of the administration of CCl_4 on the growth of rats.

The animals were grouped as follows:

Group A, 5 rats. No CCl_4. Basal diet and brewer's yeast, 1.5 per cent decreasing to 1 per cent.

Group B, 5 rats. No CCl_4. Basal diet and brewer's yeast, 8 per cent increasing to 10 per cent.

Group C, 20 rats. CCl_4. Basal diet and brewer's yeast, 8 per cent increasing to 10 per cent.

Group D, 20 rats. CCl_4. Basal diet and brewer's yeast, 1.5 per cent decreasing to 1 per cent.

The caloric intake was essentially the same for all rats and the daily food consumption per rat averaged 14 gm. This was achieved by limiting the food consumption of all animals to that of the animals in group D. The latter were fed *ad libitum*. Each animal was caged separately, fed and watered daily and weighed twice weekly.

After 11 weeks (or 22 injections of CCl_4) all rats were sacrificed after stunning and histological studies of the livers were made.

Tissues were fixed in Zenker's solution for hematoxylin and eosin and azan-carmine stains. Following CCl_4 administration, the earliest tissue changes, such as fatty degeneration and necrosis, were seen in the

* Grade 20, edible; Casein Co. of America.

central areas. Fibrosis in the liver lobule also began in the center and later extended to other areas. Therefore, the estimation of the degree of fibrosis, liver-cell vacuolization, necrosis and mitosis was made by the following methods: The central, portal and intercommunicating areas of the liver were individually graded with respect to fibrosis. The number of these anatomic areas was counted and the proportion of those which were fibrosed was determined. These were graded 1 to 4 for each anatomic area. Grade 1 signified that about 25 per cent of the specific areas was involved; grade 2, 50 per cent involvement, and grade 4, that all the specific areas were involved. The final value for fibrosis was the arithmetic sum of the grades for each anatomic area. Since three characteristics were graded; namely, central, portal and intercommunicating fibrosis, the maximum figure for a liver was 12.

The number of mitotic figures and necrotic cells per low power field was counted for three to ten fields. Vacuolization of liver cells was graded 1 to 4. Grade 1 indicated involvement of 25 per cent of the cells and 4 indicated involvement of every cell in the section. All slides were examined independently by two observers. Using the above standards, close agreement was observed. In this manner a reasonably quantitative evaluation of the lesions was made.

Results

Growth of Animals. Text-Figure 1 shows the average growth of animals of each of the four groups. No significant differences are seen between the growth curves of the two uninjected normal groups (A and B). The growth of these animals was about 1.5 gm. per day. Therefore, the ingestion of large excesses of brewer's yeast *per se* does not significantly affect the growth of normal rats.

The CCl_4 treatment depressed the growth rate of the rats in both groups C and D. However, group C, fed 8 to 10 per cent yeast, grew about twice as much as group D which was fed 1.5 to 1.0 per cent yeast. It may be concluded, therefore, that the ingestion of large amounts of brewer's yeast (8 to 10 per cent) afforded some protection against the growth-inhibiting action of CCl_4.

Histopathology of the Liver. Cirrhosis occurred in 95 per cent of the injected rats. However, in both CCl_4-injected groups there was marked variation in the liver lesions. Because of this variation the arithmetic means of the grades of the lesions were calculated. These means, recorded in Table I, are not significantly different. Figure 1 is a photomicrograph of a representative lesion for the livers showing moderate cirrhosis. The livers of the rats which did not receive CCl_4 appeared normal. In summary, it appears that in spite of its beneficial

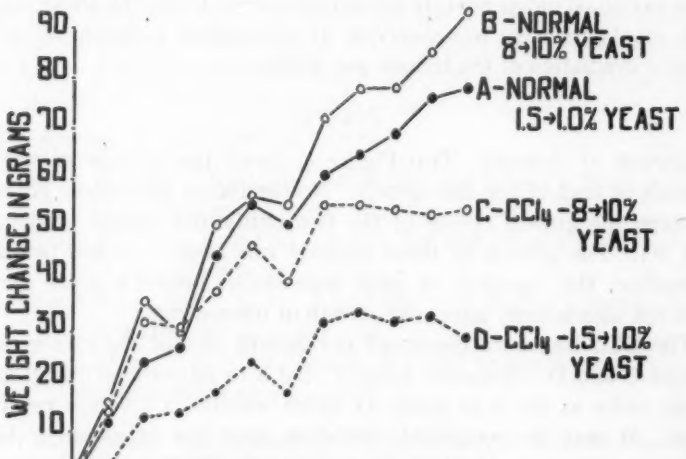
effect on the growth of CCl_4-treated rats, high yeast ingestion did not modify the severity of the liver lesion.

Fat Content of the Liver. Fat determinations on the livers were made according to the method employed by Earle and Victor.[13] From Table II it is seen that the injection of CCl_4 was associated with an

TABLE I
Liver Lesions of CCl_4-Treated Rats

Group	Dietary yeast (per cent)	Fibrosis, grade* 0–12	Vacuolization, grade* 0–4	Necrosis, no. of cells per low power field	Mitosis, no. of nuclei per low power field
Series I, C	8 –10	5.4	1.8	1.5	0.7
Series I, D	1.5 – 1.0	5.9	2.3	0.6	0.5
Series II, C′	10	7.0	1.8	†	1.1
Series II, D′	0.75– 0.2	7.8	1.8	†	1.2
Series III, E	1.77– 0.5	3.2	0.5	0	0
Series III, F	4.9 – 7.6	2.4	0.1	0	0
Series III, G	4.9 – 7.6	2.8	0.1	0	0

* The method used to estimate the grade of fibrosis and vacuolization is described in the text (see series I).
† Too many to count.



TEXT-FIGURE 1. Influence of CCl_4 and brewer's yeast on the growth of rats on isocaloric diets. Comparison of the effects of 1.5 to 1.0 per cent and of 8 to 10 per cent yeast. CCl_4 inhibited growth. Eight to 10 per cent yeast partially protected against CCl_4 inhibition of growth.

increased fat content of the liver. This confirms the findings of Winter.[14,15] However, the feeding of high yeast supplements (8 to 10 per cent) lessened to a limited, though significant degree, the lipoidosis of the liver resulting from CCl_4 administration.[16]

SERIES II

In the next set of experiments, a comparison was made of the effects of feeding amounts of yeast (0.75 to 0.2 per cent) inadequate for good growth of normal rats with the effects of feeding amounts of yeast (10 per cent) in excess of that required for good growth. As in the foregoing experiments, all animals were fed the same amounts of food.

TABLE II

Series I. Effect of CCl_4 Injections and Yeast Intake on Liver Fat Content. High Yeast Feeding Diminishes the Elevated Liver Fat of CCl_4 Cirrhosis*

Group	Yeast (per cent)	Animals	Fat (per cent)		Differences in fat content		
			Mean	Standard deviation	Compared groups	Difference of means†	P‡
A, normal	1.5–1.0	5	4.0	±0.9	A and B	1.4	1/12
B, normal	8–10	5	2.6	±0.9	C and D	*1.5*	1/88
C, CCl_4	8–10	20	6.7	±3.2	B and C	*4.1*	<1/5000
D, CCl_4	1.5–1.0	20	8.2	±3.0	A and D	*4.2*	<1/5000

* Ether soluble substances.
† Significant differences are italicized.
‡ P value of less than 1/20 is statistically significant.[17]

Methods

Male rats, 10 weeks old, from the same stock as those of the previous experiment, were employed. CCl_4 was administered in the manner previously described. The basal diet for the rats receiving 0.75 to 0.2 per cent yeast was: sucrose, 65.25 per cent; casein, 22 per cent; butter, 8 per cent, and Hawk-Oser [11] salt mixture, 4 per cent. The 10 per cent yeast diet contained 60 per cent sucrose and 18 per cent casein to correct for the caloric and protein differences due to the high yeast supplements.

The effects of these diets on the growth of similar groups of rats not receiving CCl_4 also was determined.

The animals were grouped as follows:

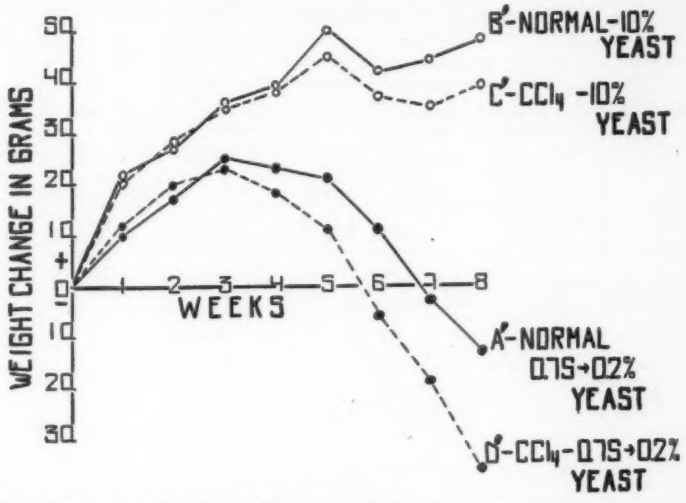
Group A′, 10 rats. No CCl_4. Basal diet and brewer's yeast, 0.75 per cent decreasing to 0.2 per cent.

Group B′, 10 rats. No CCl_4. Basal diet and brewer's yeast, 10 per cent.

Group C′, 19 rats. CCl_4. Basal diet and brewer's yeast, 10 per cent.

Group D′, 20 rats. CCl_4. Basal diet and brewer's yeast, 0.75 per cent decreasing to 0.2 per cent.

The caloric intake of all rats was similar. This was achieved by limiting the food consumption of all animals to that of the animals in group D′. These latter animals were fed *ad libitum*. The daily food intake per rat was 11 gm. during the first 4 weeks and 8 gm. during the last 4 weeks. The experiment was discontinued at the end of 8 weeks (16



TEXT-FIGURE 2. Influence of CCl_4 and brewer's yeast on the growth of rats on isocaloric diets. Comparison of the effects of 0.75 to 0.2 per cent and 10 per cent yeast. CCl_4 inhibited growth. At this level of food consumption, 10 per cent yeast protected against CCl_4 inhibition of growth.

injections) because of the cachectic state of the rats in group D′, fed inadequate yeast. Morphological changes in the livers were evaluated as previously described.

Results

Growth of Animals. In Text-Figure 2 is shown the average growth of animals of each of the four groups. That the yeast supplements of 0.75 per cent decreasing to 0.2 per cent were inadequate for growth is shown by the poor growth of the uninjected rats in group A′. Again the growth-inhibiting effect of CCl_4 was manifest. On 10 per cent yeast supplement, both the CCl_4-treated and the uninjected rats grew better than those fed inadequate yeast, in spite of the fact that the caloric intake was the same in all these groups.

Histopathology of the Liver. The mean grades of the lesions are recorded in Table I. No significant differences were found between the lesions of the rats receiving 10 per cent yeast supplements (group C′) and those receiving 0.75 per cent yeast supplements, decreasing

to 0.2 per cent (group D′). Figure 2 shows a characteristic lesion in these livers. Again, as in series I, in spite of its beneficial effect on the growth of CCl_4-treated rats, high yeast feeding did not modify the severity of the liver lesion.

TABLE III

Incidence and Degree of CCl_4 Cirrhosis at Two Levels of Food Intake as Indicated by Fibrosis

Degree of fibrosis*	Incidence per cent	
	Daily food consumption	
	Series I 14 gm. (40 rats)	Series II 11-8 gm. (39 rats)
None	5	5
Mild	25	15
Moderate	45	18
Severe	25	62

* See below for the method of grading the degree of fibrosis.

Effect of Differences in Food Consumption on CCl_4 Cirrhosis

The two series of experiments just described show that the feeding of yeast *per se* has no effect on the incidence of CCl_4 cirrhosis. Yet the average degree of fibrosis in the two series was different. Table III summarizes the incidence and severity of fibrosis in series I and II. The incidence is expressed in percentages, and the severity of fibrosis as none (0), mild (1 to 3), moderate (4 to 7), or severe (8 to 12).

Figure 1 is characteristic of the moderate hepatic fibrosis encountered in 45 per cent of the animals of series I and 18 per cent of those in series II. Figure 2 represents the severe cirrhosis found in 25 per cent of the animals in series I and 62 per cent of those in series II. The data show that the incidence of severe fibrosis was greater in series II: 62 per cent in contrast to 25 per cent in series I. These differences are greater than they appear to be, since the more severe lesions of series II occurred in animals treated with CCl_4 for a shorter time, namely 8 instead of 11 weeks, and the severity of hepatic cirrhosis is known to increase during CCl_4 treatment. The experimental conditions for these two series were the same except for the food intake. The food intake for series I was 14 gm. per day for each rat throughout the experiment; for series II it averaged 11 gm. for the first 4 weeks and 8 gm. for the last 4 weeks. At the lower level of food intake, CCl_4 produced more severe cirrhosis of the liver. It appears, therefore, that the severity of CCl_4 cirrhosis depends in part on the quantity of food consumed and not on the yeast intake.

Part II. Effect of Feeding Brewer's Yeast on the Recovery from Hepatic Cirrhosis Induced by CCl_4 in the Rat

Methods

Fifty-one male rats, 10 weeks old, of the same stock employed in Part I, were injected subcutaneously with CCl_4 in the manner previously described. The injections were made twice weekly for 8 weeks. Eight additional rats, living under the same conditions, were fed the same diet but received no CCl_4.

During the period of CCl_4 administration, all rats received the following diet: sucrose, 67 per cent; casein, 18 per cent, and Hawk-Oser[11] salt mixture, 4 per cent, containing 0.24 per cent nicotinic acid. This diet was supplemented with small amounts of brewer's yeast (Text-Fig. 3). The animals were fed *ad libitum* and the food consumption was not measured during this 8-week period. The rats were kept two in a cage and fed and watered daily.

During this 8-week period, 7 of the animals receiving CCl_4 died. Six had severe cirrhosis of the liver and 1 could not be examined because it had been eaten by its cage mate. At the end of this period 8 CCl_4-treated rats were sacrificed along with 2 untreated rats. All 8 injected rats had severe cirrhosis. The 2 uninjected rats had normal livers. From the findings of severe cirrhosis in the 8 sacrificed rats just described, it was assumed that the remaining CCl_4-treated animals also had cirrhosis of the liver. The surviving 36 CCl_4-treated rats were then studied for the influence of dietary brewer's yeast on the possible recovery from the CCl_4 cirrhosis. Below is listed the grouping of the CCl_4-treated and untreated animals (series III).

Group E, 11 rats, "cirrhotic." Basal diet* and brewer's yeast, 1.77 per cent decreasing to 0.5 per cent; fed *ad libitum*.

Group F, 15 rats, "cirrhotic." Basal diet and brewer's yeast, 4.9 per cent increasing to 7.6 per cent. Food intake limited to that of group E.

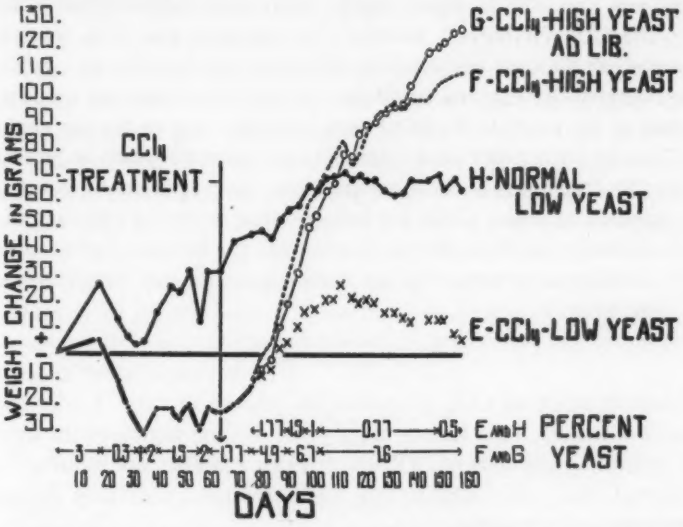
Group G, 10 rats, "cirrhotic." Basal diet and brewer's yeast, 4.9 per cent increasing to 7.6 per cent; fed *ad libitum*.

Group H, 6 rats, normal. Same diet as group E; fed *ad libitum*.

All rats were caged separately during this stage of the experiment and were fed and watered daily. Group F was fed isocalorically with group E. Group H, although fed *ad libitum*, also consumed the same amount of food as did group E. Thus groups E, F and H had the same food consumption. The food consumption of group G, fed *ad libitum*, was

* Same basal diet as described above.

25 per cent higher than that of the other groups. After 3 months of this regime all rats were sacrificed and their livers studied in the manner previously described.



TEXT-FIGURE 3. Influence of "low" and high brewer's yeast diets on growth after cessation of CCl_4 treatment. During the period of CCl_4 treatment growth was inhibited. After CCl_4 treatment was stopped, high yeast feeding accelerated growth much more than "low" yeast, in spite of isocaloric feeding.

Results

Growth of Animals. Text-Figure 3 shows the average growth of the animals described. During the 8 weeks of treatment the CCl_4-injected rats (lower line) grew less than the uninjected rats (upper line). After CCl_4 injections were discontinued all rats showed a sharp increase in the rate of growth. Those eating large amounts of yeast made a more rapid gain in weight than rats fed low yeast supplements even though the caloric intakes (excepting group G) were the same. This latter group, which was fed *ad libitum,* ate 25 per cent more food than the others, and attained a greater weight.

Histopathology of the Liver. The histological appearance of the livers is recorded in Table I. These data were derived in the manner already described.

The livers of these animals (groups E, F and G) showed far less fibrosis (Fig. 3), vacuolization and necrosis than was seen in the livers of animals sacrificed immediately after a course of CCl_4 treatment (Fig. 2). The liver scarring had almost entirely disappeared. Only

delicate strands of connective tissue remained, forming intercommunications between the liver lobules, which were regular in size and shape. The liver cells had returned to their normal size. No increase in bile ducts was present. In other words, there was considerable recovery from the CCl_4 cirrhosis 3 months after stopping the CCl_4 injections. However, in spite of the marked differences in growth, no significant morphological differences could be observed between the amount of healing of the cirrhotic livers of rats fed large (4.9 to 7.6 per cent) or small (1.77 to 0.5 per cent) amounts of brewer's yeast supplement. Under the conditions of these experiments, the 25 per cent greater food consumption of group G did not influence the degree of recovery of the cirrhotic liver. Again, as in the other series, the beneficial effect of high yeast feeding on growth was not associated with any modification of the liver lesion.

Discussion

Administration of CCl_4 depresses the growth of rats. Under conditions of isocaloric food intake, high yeast feeding improves the growth and general appearance of CCl_4-treated rats but does not influence that of normal rats. This implies that CCl_4 treatment increases the yeast requirements for growth.

In these experiments there was no correlation between the growth of animals, as influenced by yeast, and the extent of the hepatic CCl_4 lesion. Therefore, growth cannot be used as an index of the modification of this disease process. Whether or not this conclusion is applicable to other disease processes seems worthy of consideration.

Brewer's yeast *per se* had no effect on the developmental or recovery stages of CCl_4 cirrhosis. When the food consumption was diminished either by artificial restriction or by anorexia due to inadequate yeast intake, the liver lesions due to CCl_4 were more severe.

In the present studies the amount of CCl_4 injected was kept constant. It is possible that with the administration of smaller amounts of CCl_4 a specific effect upon the liver lesions could be exerted by yeast in addition to its influence on the food intake.

The finding of the lack of any specific effect of yeast on CCl_4 cirrhosis is different from the protective effect of yeast observed with other hepatotoxins.[3-5] This may be related to differences between the hepatotoxic agents. On the other hand, the reported protection may have been due in part to the appetite-stimulating effect of yeast[9,10] with resulting increase in the food intake. From the present studies it would seem essential to control the food intake in this type of investigation.

Summary and Conclusions

1. The effect of different levels of brewer's yeast ingestion on the hepatic cirrhosis produced by CCl_4 injections was studied in isocalorically fed rats. The lesions were studied both during the developing and healing stages of the disease.

2. The development of and recovery from cirrhosis of the liver was essentially the same whether amounts of yeast fed were grossly inadequate, adequate or in excess of the requirements for normal growth.

3. The addition of large amounts of brewer's yeast to the basal diet overcame in part a growth-inhibiting effect caused by CCl_4 injections.

4. The addition of large amounts of brewer's yeast to the basal diet decreased the lipoidosis of the liver caused by CCl_4 injections.

5. In contrast to the yeast intake, the food consumption modified the severity of the cirrhosis. Those fed 8 to 11 gm. of food daily had more severe liver lesions than those fed 14 gm. daily, regardless of the amount of yeast supplement.

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ADDENDUM

Since this paper was submitted, a number of papers have appeared which describe the experimental production of cirrhosis of the liver by means of deficient diets:

Blumberg, Harold, and McCollum, E. V. The prevention by choline of liver cirrhosis in rats on high fat, low protein diets. *Science*, 1941, **93**, 598–599.
Daft, F. S.; Sebrell, W. H., and Lillie, R. D. Production and apparent prevention of a dietary liver cirrhosis in rats. *Proc. Soc. Exper. Biol. & Med.*, 1941, **48**, 228–229.
György, Paul, and Goldblatt, Harry. Observations on the conditions of dietary hepatic injury (necrosis, cirrhosis) in rats. *J. Exper. Med.*, 1942, **75**, 355–368.
Webster, Graham. Dietary liver disease in rats. (Abstract.) *J. Clin. Investigation*, 1941, **20**, 440.

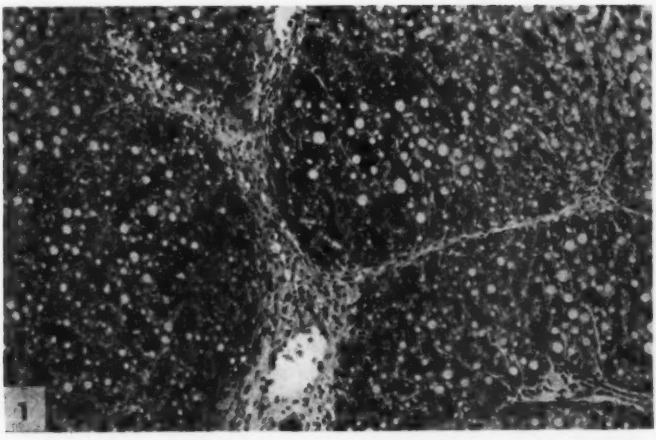
DESCRIPTION OF PLATE

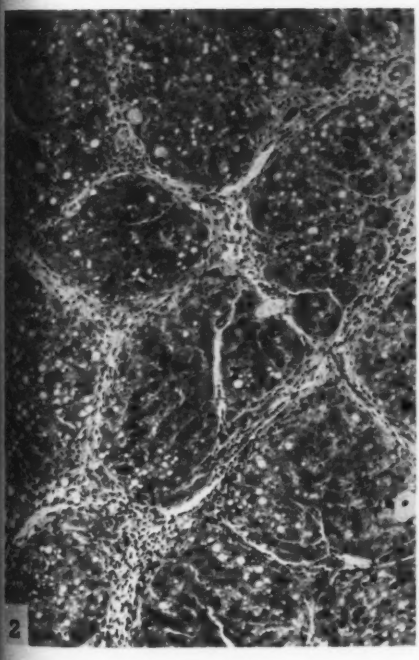
PLATE 103

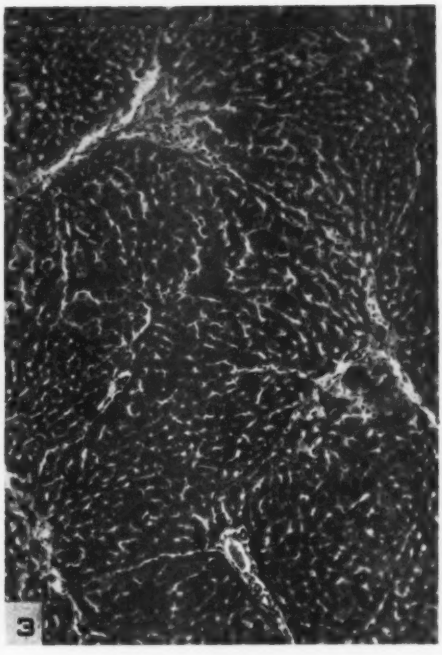
FIG. 1. Moderate CCl_4 cirrhosis. Fibrosis about the central areas with some extension of the scars to adjacent central areas. Hypertrophy and vacuolization of the liver cells. Hematoxylin and eosin stain. × 100.

FIG. 2. Severe CCl_4 cirrhosis. Intercommunicating bands of dense scar tissue are seen, with newly formed bile ducts and hypertrophy and vacuolization of the liver cells. Hematoxylin and eosin stain. × 100.

FIG. 3. Healing CCl_4 cirrhosis. Delicate connective tissue strands outlining the liver lobules by communication between central and portal areas. Liver cells of normal size. Hematoxylin and eosin stain. × 100.







Earle, Patek and Victor Effects of Yeast on Hepatic Cirrhosis

PRIMARY CARCINOMA OF THE LIVER *

CHOLANGIOMA IN HEPATOLITHIASIS

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From the standpoint of its pathogenesis, primary carcinoma of the liver, including both hepatoma and cholangioma, has been linked to a number of preëxisting hepatic lesions. Among these are cirrhosis (Laënnec's, pigment, toxic, obstructive biliary), parasitic infestation (clonorchiosis, distomiasis, schistosomiasis, echinococcosis), syphilis, congenital rests, chronic venous congestion, cholangitis and hepatolithiasis.

In the following two cases we report the rare occurrence of cholangioma, discovered as an incidental finding at autopsy, with hepatolithiasis associated with cholangitis and cholestasis.

REPORTS OF CASES

CASE 1

M. O., a white married housewife, 61 years of age, was admitted to the Buffalo General Hospital (service of Dr. Clayton W. Greene and Dr. Benjamin Jacobson) on September 23, 1938, because of stupor of 4 days' duration. She died in 12 hours.

In December, 1934, the patient fell down two steps and injured her back. At this time diabetes mellitus was diagnosed. In March, 1936, the patient was admitted to the hospital for pain in the knees. Upon discharge, the diagnoses were rheumatoid arthritis; diabetes mellitus; calcification of the arteries of the feet; compression of the twelfth dorsal vertebra and lipping of the eleventh, twelfth dorsal and first lumbar vertebrae; incisional hernia. There was no history of jaundice. On September 19, 1938, the patient became stuporous.

Examination. The temperature was 101.2° F. per rectum. The patient was stuporous but able to carry out orders. The eyeballs were soft. The abdomen showed an incisional hernia. Urinalysis gave a positive Benedict reaction. Before death the temperature reached 106° F. per rectum; cyanosis became prominent.

Autopsy Findings

The autopsy was performed 2 hours after death. The pathologic diagnoses were: early diffuse and patchy pneumonia of the right middle and lower lobes; inflammatory hyperemia of the right upper lobe; hepatolithiasis with dilatation, in places localized, of intrahepatic bile ducts containing thick, brown, sandy, mucopurulent bile and showing in some ducts papillary formations of the lining; a small papillary and solid bile duct carcinoma (cholangioma) of right lobe of liver; chronic cholangitis of extrahepatic bile ducts with choledocholithiasis

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and marked dilatation of the lumina; chronic cholecystitis with cholelithiasis; dilatation of pancreatic duct with atrophy of pancreatic tissue, fatty infiltration and interstitial fibrosis; hypertrophy of islets of Langerhans; postmortem blood sugar, 266 mg. per 100 cc., and CO_2-combining power, 16 mm. of mercury (diabetes mellitus); atherosclerosis; atrophy of the brain (950 gm.); myocardial fibrosis; compression of anterior surfaces of bodies of eleventh and twelfth dorsal vertebrae; leukoplakia of esophagus; healed laceration of cervix; fibrous pleurisy; incisional hernia; absence of appendix.

Gross Findings in Liver and Pancreatico-Biliary Tract

When the abdominal cavity was opened, the superior and anterior surfaces of the liver were found to be adherent to the inferior surface of the right hemidiaphragm by fibrous adhesions. Upon separation of these, the hepatic surfaces showed circumscribed, white-yellow, opaque elevations which measured up to 3.5 cm. in diameter. Palpation of these elevations elicited crepitus. The liver was reduced in size. The right lobe measured 17 by 15 by 8.5 cm.; it possessed an accessory cleft; the left lobe measured only 5 by 6 by 3 cm. On section, the cut surface revealed marked dilatation of the larger intrahepatic bile ducts with the formation of cavities along the course of the ducts. The white-yellow elevations, noted externally, corresponded to walls of cavities which reached the surface of the liver. In the left lobe such dilatation and cavitation was so marked that the parenchyma had almost disappeared from compression atrophy.

The dilated ducts and cavities measured up to 3.5 by 1 cm. Their walls were thickened. The content was composed of thick, brown, mucopurulent, sandy bile and stones. Smears revealed a variety of organisms, chiefly gram-negative bacilli and gram-positive cocci. For the most part, the stones were soft, brown and easily pulverized. They ranged in size from that of a pea to a pistachio nut. Multiple small faceted stones were also found in ducts. Chemical analysis of stones was positive for bile pigment and calcium. In some ducts and cavities stones fitted tightly. While in the main it appeared atrophic, the lining of several ducts and cavities in scattered locations demonstrated soft, low papillary formations without invasion of walls. The hepatic parenchyma was not icteric; the smaller portobiliary spaces were prominent.

On one gross section through the liver, there was discovered, practically by accident, in the anterosuperior medial portion of the right lobe (Fig. 1), a ductular cavity 1.8 by 1.8 cm. which presented, proximal to the site of lodgment of a large stone, a white, papillary growth into the lumen of greater extent than had been previously seen. On

gross serial sections this papillary growth was found to fill completely the lumen of the duct, where it was less dilated proximal to the stone, and apparently to invade the wall. Here, surrounding the duct, especially anteriorly and medially, were several solid, white-yellow nodules, separated by gray-yellow septa. These nodules measured, with the duct included, 2.5 by 2.5 cm.

The capsule of the liver above the gallbladder was fibrosed. The gallbladder was dilated, measuring 13 by 10 cm. The serosa was fairly smooth. The wall measured 1 mm. in thickness. The mucosa was smooth, atrophic, showed no bile imbibition and its content was mucoid. The extrahepatic bile ducts were distinctly dilated: hepatic duct, 3 cm. in circumference; cystic duct, 1.7 cm., and common bile duct, 3 cm. The lining of the extrahepatic bile ducts was smooth. The walls were opaque and thickened. The bile was mucopurulent, brown and thick. The stones found in the extrahepatic bile ducts and gallbladder were small, averaging 0.9 cm. in diameter and were of the faceted, combination type.

The pancreatic duct opened above the common bile duct. In the head and body it was dilated to 1 cm. in circumference and contained brown gravel. The parenchyma of the pancreas was atrophic. The lymph nodes were free of metastases.

Microscopic Findings in Liver

The findings in the bile ducts were varied. In sections taken from the right lobe, the small bile ducts were lined by a single layer of high cuboidal epithelium. There was a distinct periductular fibrosis. Cellular infiltration in the portobiliary spaces was slight or minimal. The dilated ducts and cystic-like cavities from both lobes revealed atrophy or absence of epithelial lining. The walls were markedly thickened and fibrosed; they were only slightly inflamed.

The white papillary formations noted grossly in several dilated ducts and cavities distant from the tumor were of different types. In two ducts they were dependent upon a papillary and polypous inflammatory hypertrophy of the mucosa. Here glandular epithelium showed mitotic figures and large dark nuclei. In other ducts adenomatous hyperplasia of typical and also of atypical nature had occurred. In one, the basis for the papillary formation was papillomatous and adenomatous proliferation of the mucosa and wall. Here proliferations of the lining were rather cellular and contained little stroma. In a number of epithelial cells mitotic figures and atypical nuclei were found. Invasion of the wall was not demonstrated.

In contrast to the dilated ducts and cavities with atrophic or absent

lining, the ducts with epithelial and glandular proliferations were markedly inflamed. In the mucosa and wall there was distinct infiltration of neutrophils, mononuclear cells and plasma cells. Fibrosis of the wall was marked. Bile pigment was inspissated. Some of the small bile ducts in the vicinity of the carcinomatous site contained leukocytes, desquamated cells and granular débris. Rarely bile pigment was seen in the lumina. The lining membrane, where still preserved, was single-layered, with occasional hyperchromatic nuclei. The walls were infiltrated with cells of inflammatory origin. The portobiliary stroma was increased in amount and infiltrated with neutrophils, round cells and plasma cells.

On the medial margin of the carcinomatous site, three medium-sized and larger ducts, in addition to having exudate and desquamated cells in the lumen and active chronic inflammation in the wall, revealed marked papillomatous growth of the mucosa which practically filled the lumina. In these ducts there was a gradation in the character of the epithelial proliferation from a more or less typical form to atypical proliferation with large hyperchromatic nuclei, mitotic figures and early invasion.

The large duct in the central portion of the carcinomatous site showed, besides chronic nonspecific inflammation, marked papillomatous proliferation, and here in places the epithelial growth was altogether medullary and atypical. In the wall there was epithelial invasion of both solid and glandular types. The picture in this duct was that of a papillary, solid and glandular carcinoma (Fig. 2).

The nodules surrounding the duct just described were composed of solid carcinomatous alveoli and trabeculae. The cells were chiefly polyhedral with rather distinct outline and blue-staining cytoplasm. Nuclei were vesicular, oval, or round. Chromatin was arranged in small dots or nucleoli. Many mitotic figures and atypical nuclei were seen. No sinusoids ran between the cells. Alveoli and trabeculae within the nodules were separated by thin-walled vessels or by a slight amount of connective tissue. There were no multinucleated giant cells. The large nodules were separated by inflamed fibrous tissue. Throughout the solid alveoli and trabeculae cells tended to take cuboidal and columnar shapes and to form ductular and glandular structures suggestive of bile ducts. Lymphatic and perineural spaces were invaded by the tumor. In one area of solid carcinoma necrosis and hemorrhage had occurred. The blood vessels were dilated. In the stroma of the solid tumor macrophages contained brown pigment. On low power examination the carcinomatous site appeared well outlined. In places it was separated from the surrounding liver by connective tissue. On high

power examination, in certain areas cords of carcinoma cells combined and merged with surrounding liver cells which showed large dark nuclei.

In the hepatic parenchyma the lobular markings were preserved. There were distinct regressive lesions in liver cords—hydropic and granular degeneration, necrosis of cells and dissociation of cords—distributed in both central and peripheral zones. Cells with large and double nuclei were seen. Surrounding dilated ducts and cavities, the hepatic parenchyma was compressed and atrophic. The walls of small arteries were thickened and hyalinized. In medium-sized arteries intimal sclerosis was present. In large vessels the internal elastic layer had become calcified in part. The lymph nodes showed chronic, nonspecific lymphadenitis but no metastases.

CASE 2

W. M., a white man, 62 years of age, was admitted to the Niagara Falls Memorial Hospital (service of Dr. George Stoll) on December 27, 1939, with chills and fever. He died January 15, 1940.

For 3 weeks the patient had suffered with epigastric pain. One day before admission he developed fever and chills. There were abdominal distention and vomiting. About 1 year previously the patient had had two operations on his biliary tract.

Examination. The skin was icteric. Râles were heard in the left lower chest. The heart was slightly enlarged. A systolic murmur was heard at the mitral area. The abdomen, which showed a scar in the right upper quadrant, was distended and tympanitic. The liver seemed to be enlarged. The left arm and leg were paralyzed.

Laboratory studies disclosed: hemoglobin, 55 (Newcomer); red blood cells, 2,850,000 per cmm.; white blood cells, 21,200 per cmm. with 88 per cent neutrophils, 10 per cent lymphocytes and 2 per cent transitionals; icteric index, 9. Urine: specific gravity, 1008; albumin, 1 plus; few red blood cells; many white blood cells. Blood culture was negative. The temperature ranged from 98.6° to 106° F. Terminally, cough and cyanosis appeared.

Autopsy Findings

The autopsy was performed 1 hour after death. The pathologic diagnoses were: status following hepaticoduodenostomy; abdominal scars; choledocholithiasis of common bile, cystic and hepatic ducts; chronic cholecystitis; hepatolithiasis, chronic cholangitis of hepatic and intrahepatic ducts; small cholangioma of right lobe of liver; purulent thrombophlebitis of portal and superior mesenteric veins; multiple pylephlebitic abscesses of liver; metastatic abscesses of left lung with fibrinous pleuritis; purulent bronchiolitis; diffuse and confluent bronchopneumonia of right lower lobe and slight serofibrinous pleuritis; pulmonary embolism and thrombosis of right upper lobe; chronic splenitis; leiomyoma of esophagus; solitary cyst of left kidney; chronic hemorrhagic cystitis; chronic prostatitis with atypical

epithelial proliferation; slight myocardial fibrosis; ascites; edema of legs. Smears and cultures from liver, portal vein and lungs revealed Friedländer's bacillus.

Gross Findings in Liver and Pancreatico-Biliary Tract

When the peritoneal cavity was opened, anatomic relations in the right upper quadrant could not easily be made out. There were marked fibrous adhesions involving the parietal wall behind the abdominal scar, the stomach, the duodenum and the liver, which was also adherent to the inferior surface of the right hemidiaphragm. The hepatoduodenal ligament was shortened. The pylorus was patent. The first portion of the duodenum was dilated. In its anterosuperior margin, 6.6 cm. from the pylorus, was an opening 0.8 cm. in diameter, from which escaped thick pus, bile and gravel. This duodenal opening communicated with the main hepatic duct which was 2.2 cm. in length and 1.8 cm. in circumference. The ductular wall was distinctly thickened. The lumen contained mucopurulent material, biliary gravel and stones.

The right and left hepatic ducts showed similar findings in the walls and lumina. They were dilated and apparently shortened. The common bile duct measured 8.2 cm. in length. It was dilated to 2.5 cm. in circumference and the wall was slightly thickened. The lumen contained multiple combination stones. The cystic duct, 1.5 cm. long, was contracted upon stones. The gallbladder was contracted, measuring 4 by 2.2 cm. Its wall was 0.2 cm. thick. There were no stones in it.

The right lobe of the liver measured 18 by 14 by 6 cm.; the left lobe, 16 by 7 by 7 cm. The right lobe appeared deformed. Shining through the thickened capsule of the liver were a number of soft yellow nodules. On section, these, as well as other nodules in the hepatic parenchyma, proved to be abscesses with walls of lipoid-containing granulations. The pus was non-fetid, thick and mucoid in character. The intrahepatic bile ducts were dilated. They contained pus, bile, gravel and combination stones of cholesterol-pigment type, and in some ducts, stones were lodged tightly. Stones were more numerous in the right lobe than in the left. On section through the right lobe of the liver there was discovered a dilated, thickened duct which contained stones but which was also filled with soft yellow, papillary growth. Similar growth was found in other ducts in this region. The entire area was about 2.5 cm. in diameter. The growth reached the posterior surface of the liver. It could be made out grossly that the papillary growth did not originate in the gallbladder or in extrahepatic bile ducts. No metastases were found in the nodes.

Microscopic Findings in Liver

There was a purulent thrombopylephlebitis. The huge abscesses with much fibrin were of pylephlebitic origin and some of these were multilocular. They were surrounded by thin walls of connective tissue infiltrated with round cells, neutrophils, plasma cells and eosinophils. The adjacent liver was compressed. The hepatic parenchyma in both lobes showed distinct obstructive-infective biliary cirrhosis. There was distinct icterus, particularly in the central zones, with bile in the canaliculi. The hepatic cells showed marked regressive changes. The lobules were small and the portobiliary tissue was increased in amount, with infiltration of neutrophils and round cells. Bile ducts were more numerous than normal and they were dilated. Their lumina contained bile pigment and concrements. The arteries showed intimal thickening. In the carcinomatous site, hepatic architecture no longer remained and findings in the bile ducts varied. Some ducts were dilated by impacted cholesterol pigment stones. The epithelium was atrophic or desquamated; it was also atypical. Other dilated ducts contained purulent exudate in the lumina, with red blood cells and desquamated epithelium. Bile pigment was also present. Ductular walls were fibrosed; they were infiltrated with neutrophils, plasma cells and round cells.

One large duct near the central portion of the carcinoma showed exudate and "stone" in the lumen and inflammation in the wall. For the most part, the lining of this duct was made up of a single layer of typical columnar cells. In one part of the lining, however, there was marked atypical proliferation with papillary formations and large hyperchromatic nuclei. In this area there was also invasion of the wall. Similar atypical epithelial proliferation with invasion, associated with marked inflammation, was found in other large ducts (Fig. 3). The invading carcinoma represented various types of growth. For the main, it was a mature, papillary adenocarcinoma. In certain areas it was mucin-producing. In other places it was immature in character and contained giant cells. The surrounding liver tissue was invaded. Necrosis was moderate in amount. There was a moderate scirrhous reaction with inflammation and lipoid resorption. Perineural and lymphatic spaces and nerves were invaded. The tumor reached the capsule of the liver and the gallbladder bed. There was a marked chronic perihepatitis. Lymph nodes showed no metastases.

Comment

The question arises as to the possibility of a pathogenetic relationship between hepatolithiasis, with concomitant chronic biliary infection and cholestasis, and the primary carcinoma of the liver. In answering

this question, our cases have particular significance because in each the carcinoma was only an incidental finding at autopsy. It gave no clinical symptoms. Its size was relatively small. Its origin and spread could still be fairly well inferred and traced. It produced no metastases.

Stones, along with the accompanying cholestasis and chronic inflammation, have been accepted as important factors in the development of carcinoma of the gallbladder.[1] Ewing[2] stated that in addition "to mechanical effect of calculi, presence of cholesterol and irritative and digestive action of the bile" play major pathogenetic rôles.

How far can the relation which exists between stones with cholestasis and chronic inflammation and carcinoma of the gallbladder be applied by analogy to carcinoma of the liver? In our cases intrahepatic bile ducts were dilated along their course into inflamed, fibrosed, cyst-like structures which contained stones and infected, thick, sandy bile. In case 1 a typical non-invasive, papillomatous and adenomatous proliferation occurred along with inflammation and lodgment of stones in ducts distant from the carcinomatous site. In both cases the carcinoma itself was histologically a cholangioma which could be directly related topographically and pathogenetically to an intrahepatic calculous cholangitis with marked papillary and glandular proliferation and pericholangitis (biliary cirrhosis).

Yamigawa[3] cited six cases (G. Kika) of carcinoma of the large intrahepatic bile ducts combined with cholangitis and pericholangitis exhibiting papillomatous and adenomatous hyperplasia of mucosa and wall. In one of these the inflammation and hyperplasia were caused by impaction of a biliary stone. Dahl[4] reported a case of cholangioma in paratyphoid cholangitis, cholecystitis, cholelithiasis and biliary cirrhosis, but in this instance we feel that it is difficult to evaluate pathogenetic relations. Rufanov[5] stated that cases of neoplasm of the ducts have been seen in hepatolithiasis, but he made no specific reference to a report of carcinoma of intrahepatic bile ducts associated with liver stones. According to Ewing,[2] carcinoma of intrahepatic bile ducts commonly yields a history of severe disturbance of evacuation of bile.

Certain experiments on the effects of different degrees of cholestasis on the lining of intrahepatic bile ducts are available. Partial ligation of bile ducts in dogs produces degeneration and desquamation of the epithelium in the large intrahepatic bile ducts.[6] Following complete aseptic ligation of the common bile duct or of an hepatic duct,[7,8] there develops high-grade papillary and adenomatous proliferation of the mucosa of medium-sized and large intrahepatic bile ducts with hyperchromatic nuclei, mitotic figures, inflammation and periductular fibrosis. The small ducts also proliferate. Subsequently the ducts undergo

destruction or dilatation with flattening of the epithelium. Inasmuch as chemical analysis of bile from intrahepatic ducts was not made in our cases, we can offer no comment on specific changes in the composition of that bile which was not being evacuated normally from a liver with parenchymal damage, nor on the possible relation between these changes and the pathologic findings.

Summary

Primary bile duct carcinomas (cholangiomas) of the liver associated with hepatolithiasis, cholangitis and cholestasis were discovered as incidental findings at autopsy in a diabetic woman, 61 years old, and in a man, 62 years old, with history of biliary tract disease. The question of the pathogenetic relationship of hepatolithiasis with cholangitis and cholestasis to primary bile duct carcinoma of the liver is discussed.

Note: We are indebted to Dr. Kornel Terplan for his helpful criticism and to Dr. Clayton W. Greene and Dr. George Stoll for their permission to transcribe the clinical records.

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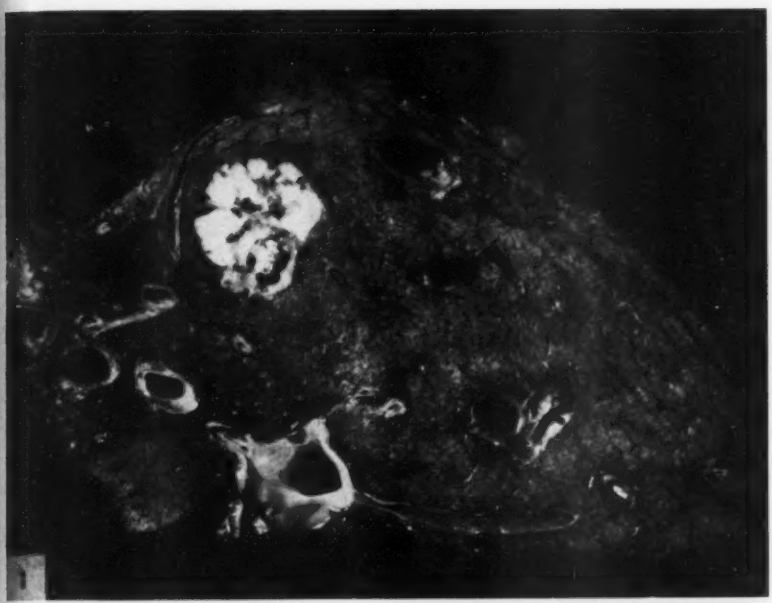
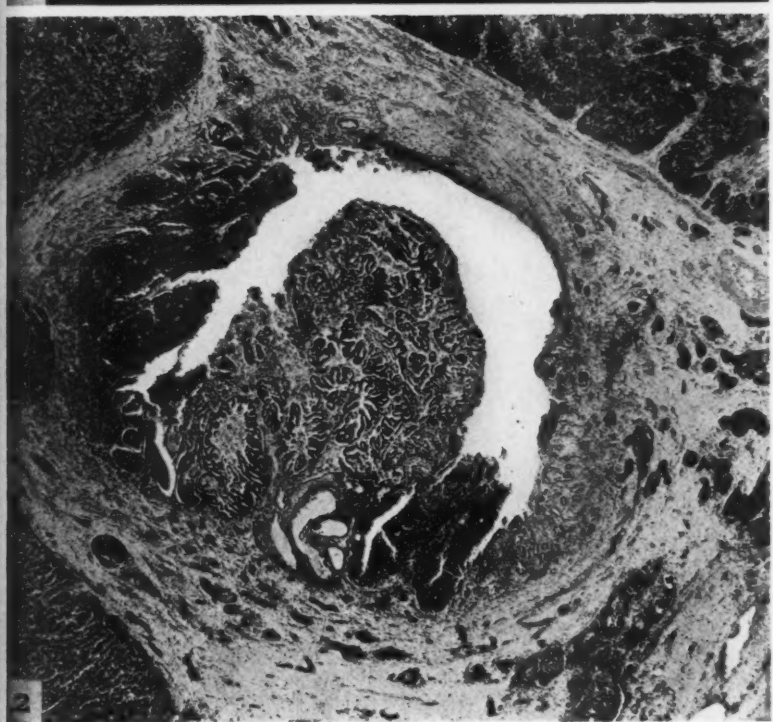
DESCRIPTION OF PLATES

PLATE 104

FIG. 1. Case 1. Gross appearance of liver with small carcinoma of bile duct origin (cholangioma) and dilated bile ducts.

FIG. 2. Case 1. Low power view of cholangioma showing papillary, glandular and solid types. × 10.





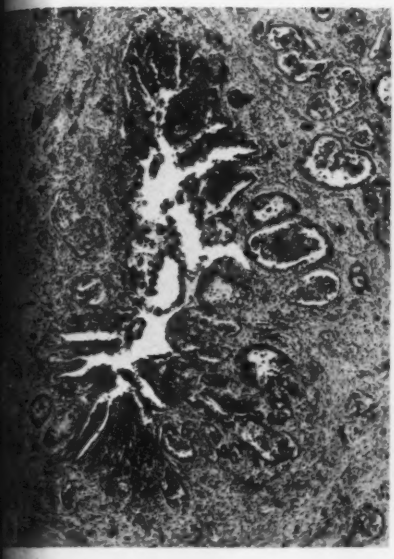
ES and MacCallum Cholangioma in Hepatolithiasis

PLATE 105

FIG. 3. Case 2. Low power view of bile duct showing papillary adenocarcinoma and inflammation. × 17.

FIG. 4. Case 2. Low power view of dilated bile duct with concrement. × 17.

FIG. 5. Case 2. High power view of bile duct showing invading adenocarcinoma. × 136.

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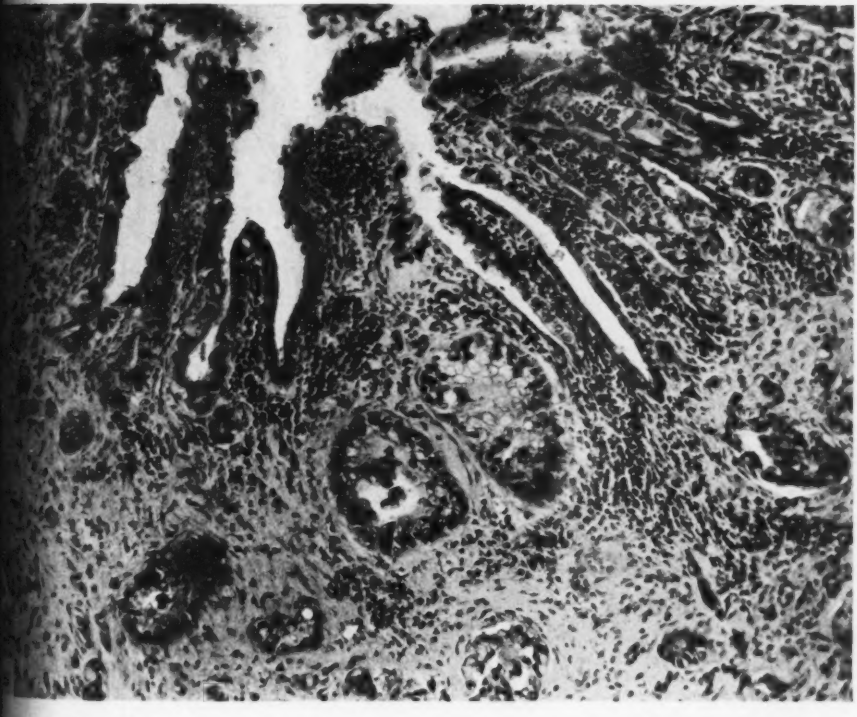

BONE-MARROW CHANGES PRODUCED BY SPECIFIC ANTIBODIES *

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Animals injected repeatedly with heterologous tissue extracts are capable of producing antibodies against the original extracts. Such antibodies may be demonstrated in a number of ways. With this organ extract (antigen)-antibody technic, Masugi [1] produced progressively damaging lesions in the liver and kidneys. Antiplatelet antibodies, which reduced circulating platelets to zero, and purpura were demonstrated in animals by Ledingham.[2] Similarly acting antilymphocytic and antileukocytic serum has been made by Chew, Stephens and Lawrence.[3,4] Clinically and in experimental hemolytic anemia, Damesheck and Schwartz [5] showed that erythrocytes may be destroyed by anti-erythrocyte antibodies. Antibodies capable of producing abortion, apparently by their specific action on the placenta, were demonstrated in rabbits and in two human cases of spontaneous separation of the placenta by Cohen and Nedzel.[6] Precipitin methods have demonstrated antibodies against lung (Salfeld and Weichsel [7]). Pfeiffer,[8] as early as 1905, made the first recorded attempt to produce antitissue antibodies; he utilized spermatozoa. Burky,[9] using sensitivity methods, showed specific antibodies for lens and muscle. Bailey and Gardner,[10] by passive anaphylaxis, produced tissue specific antibodies for brain and medullated nerves. Although Schwentker and Rivers [11] were not altogether clear about the mechanism which produced extensive structural damage in the brains of rabbits by the injection of extracts of homologous brain, it seems likely that it was on an antigen-antibody basis.

The present study was essayed in order to determine the effect on the blood picture and bone marrow following single and multiple injections of heterologous bone-marrow antibodies.

MATERIAL AND METHOD OF PREPARATION

Macerated rabbit bone marrow was obtained by dissecting and crushing open heads of the long bones, flat bones, sternum, ribs and vertebral bodies and grinding with mortar and pestle. Slightly more than an equal quantity of distilled water was added and to this the same amount of cold ethyl ether. This was repeatedly shaken in the

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cold for a period of 4 hours and the ether-fat fraction allowed to separate. The water extract was then drawn off and its protein rapidly precipitated by adding to 20 parts of the water extract, 80 parts of cold c. p. acetone. The precipitate was quickly centrifuged and the acetone decanted. The precipitate was then put into solution in a minimum of distilled water. This gave a cloudy gray-brown solution which was used for injections. Fresh solutions were made every 2 weeks. One to 2 ml. of this solution was injected intramuscularly into guinea pigs for 16 bi-weekly injections. The guinea pigs were bled out by cardiac puncture 10 days after their last injection. The pooled serum from their clotted blood was stored in an ice box and used as soon as possible.

The guinea pig antibone-marrow serum was injected in amounts ranging from 5 to 10 ml. into the lateral ear veins of rabbits whose blood picture previously had been proven to be normal and whose leukocyte count directly prior to injection was less than 11,000, with a normal differential count. Following the antibody injections, counts were done at 5, 10 and 15 minutes and 24 hours following injection. One group of animals received repeated antibody injections over a period of 2 months. On these, daily counts and differential studies were done. Biopsies were taken from time to time on both groups and autopsies finally performed. The usual routine autopsy examinations were done with particular reference to the microscopic sections of the upper femoral, pelvic and sternal bone marrow and spleen.

Results

Peripheral Blood Studies

All 12 animals which received a single injection of antibone-marrow serum showed an immediate leukopenia caused by reduction in circulating myeloid cells. The polymorphonuclear leukocytes may be driven to zero within 15 minutes following the injection. Thus there is a relative lymphocytosis which persists for 24 hours even though the total count has returned to near normal at that time. Other blood changes were not found before the animals were sacrificed for microscopic study at 72 hours. The results of the peripheral blood studies obtained in these experiments are summarized in Table I.

A second group of 12 animals were injected at intervals of 1 to 6 days over a period varying from 1 week to 2 months. The amounts injected varied from 0.5 to 5 ml. of antibone-marrow serum. In spite of considerable variation in time intervals of injection and in the amount injected, the peripheral blood studies were consistent with

those seen in the earlier group. The sharp reductions still occurred when antileukocyte serum was injected, with a relative lymphocytosis. However, when the injections were repeated over a period of time, the animals developed, in addition to, and following the leukopenia, a slight leukocytosis. Reductions in hemoglobin were common following a number of injections. One such animal, rabbit A in Table II, showed a profound anemia. Table II summarizes the peripheral blood studies of 2 animals which received repeated injections.

TABLE I

Total Leukocyte Counts

Animal	Pre-injection count (15 min.)	Amount injected (ml.)	Count after injection		
			5 min.	15 min.	24 hrs.
A	11,000	10	4,900	3,700	12,700
B	10,300	10	3,000	4,100	13,500
C	10,500	10	3,600	4,000	8,150
D	10,600	5	2,400	3,200	10,600
E	9,300	5	2,600	5,000	12,400
F	6,000	5		5,200	13,100
G	10,200	5	<100	4,300	11,000
H	8,000	5	4,200	1,500	8,600
I	7,600	5	3,500	4,300	11,300
J	9,100	8	<200	<200	9,100
K	8,800	8		5,000	10,200
L	10,800	8	3,900	4,600	13,000
Total averages for 12 animals given above	9,300	7	28,000	3,700	11,100

Typical Differential Count in Per Cent

	Polymorpho-nuclears	Basophils	Lymphocytes
5 min. after injection	5	1	92
15 min. after injection	0	0	97
24 min. after injection	14	1	85

Gross Bone-Marrow Findings

All of the bone marrow examined in the animals which received a single injection showed an active hyperemia and scattered, minute petechiae. In the animals which were injected repeatedly, hemorrhages were not prominent. Instead, there were many small yellowish gray flecks, apparently of necrosis. Also, there were larger gelatinous areas, and some areas of the bone, particularly near the epiphyses, were quite fibrotic. The spleens in all of the animals of this last group were greatly enlarged and on section showed a hyperplastic gray pulp.

Histologic Findings

The microscopic picture found in those animals which had received a single injection differed considerably from those which had a number of injections. By studying these and the animals which had received multiple injections, four well defined phases were found. The first phase was marked by capillary dilatation, hemorrhage and fresh areas of cellular necrosis. This occurred after any single injection

TABLE II

Peripheral Blood Following Repeated Injections

	Days of experiment	Pre-injection count	Amount injected (ml.)	Post-injection count (15 min.)	Hemoglobin
Animal A	1	11,400	10	4,400	
	3	6,150			
	4	10,850			5.5 gm. (36%)
	7	12,650			4.0 gm. (28%)
	9	8,250			3.0 gm. (21%)
	10	9,800	7	6,600	2.8 gm. (20%)
	11	11,650			2.8 gm. (20%)
	15	10,550			5.0 gm. (35%)
	19	7,300	3	7,100	
	21	7,050			
	24	10,950			9.0 gm. (58%)
	28	5,950	3	4,500	
	29	9,300	3	6,050	
	59*	14,400	3		
	61	5,200 (sacrificed)			
Animal B	1*	10,700	2	3,200	
	2	22,400			
	3				
	4	8,800	3	2,100	
	5	8,600			
	7	12,100 (sacrificed)			

* Biopsy.

regardless of whether or not there had been previous ones. The areas of necrosis occurred indiscriminately in all bones studied and involved from a few cells to an area as large as 300 to 500 μ across. All of the cells in the involved area were damaged, although some may have been capable of surviving. Stromal and parenchymal cells were equally damaged. The parenchymal cells showed partial loss of the cytoplasm and poor staining of the remainder, with hyperchromia of the nuclei. Cells in the areas of necrosis in animals receiving multiple injections showed even more irreparable cytoplasmic and nuclear damage (Fig. 1). The second phase was marked by regrowth which quickly passed into the third phase of hyperplasia (Fig. 2). Hyperplastic areas were found in all of the animals receiving multiple injections. Even these

were not immune to necrosis and, following a new antibody injection, were necrosed as extensively as was normal bone marrow. The fourth phase consisted of proliferation of fibrous connective tissue, scarring and cyst formation (Figs. 3 and 4). Early in the fourth phase young fibrous connective tissue growth was evident. This seemed to arise from the endosteal cells (Fig. 3). The heaviest connective tissue growth was found near the epiphyses. Cysts were found most often in the long bones. The substance in them stained a light pink.

The larger doses of antiserum produced more extensive changes than did smaller amounts.

Discussion

It seems clear that a single injection of specific antibody produces a direct change in the bone marrow which is reflected in the peripheral blood. This change, consisting of an indiscriminate necrosis of bone-marrow elements, is accentuated by repeated injections so that the end-picture is similar to that seen in osteitis fibrosa of humans. The peripheral blood picture shows a consistent primary leukopenia followed in later injections by a slight leukocytosis. It is not at present possible to state the mechanism of the bone-marrow necrosis. Its rapidity of occurrence is suggestive of direct damage by antibodies. However, Kay [12] has proposed a much more complicated mechanism in experimental antibody nephritis.

The usual occurrence of necrosis in the central portions of the bone marrow may be brought about because the perforating arteries supply the cortical bone marrow first and thus the central bone marrow is relatively anoxemic. Hyperplasia and regrowth adequately explain the leukocytosis encountered late in the protracted experiments. The appearance of hyperplastic areas along the periphery may mean that the bone marrow regenerates from this region. If this be true, we have another clue as to how bone marrow grows following damage in various conditions.

Summary

In summary, a method is given for obtaining antibodies against rabbit bone marrow. The injection of these specific antibodies causes an immediate reduction in circulating cells of bone marrow origin. The antibody acts not only on the circulating leukocytes but also directly on the bone marrow, producing in it hemorrhage, areas of acute necrosis, hyperplasia of the remaining bone marrow and finally extensive fibrosis and cyst formation. There is similarity of the experimental fibrotic lesions to those found in humans with fibrosa cystica.

Note: I am indebted to Edward Moore, of the Albany Medical College, for the photomicrographs.

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DESCRIPTION OF PLATES

Plate 106

Fig. 1. Pelvic bone marrow from an animal which had received three weekly injections of antibone-marrow antibody. There is widespread destruction of the cytoplasm and understaining of the nuclei. × 540.

Fig. 2. Sternal bone marrow removed from an animal which had received two antibone-marrow antibody injections. There is active hyperemia and extensive, early marrow-cell hyperplasia. × 540.

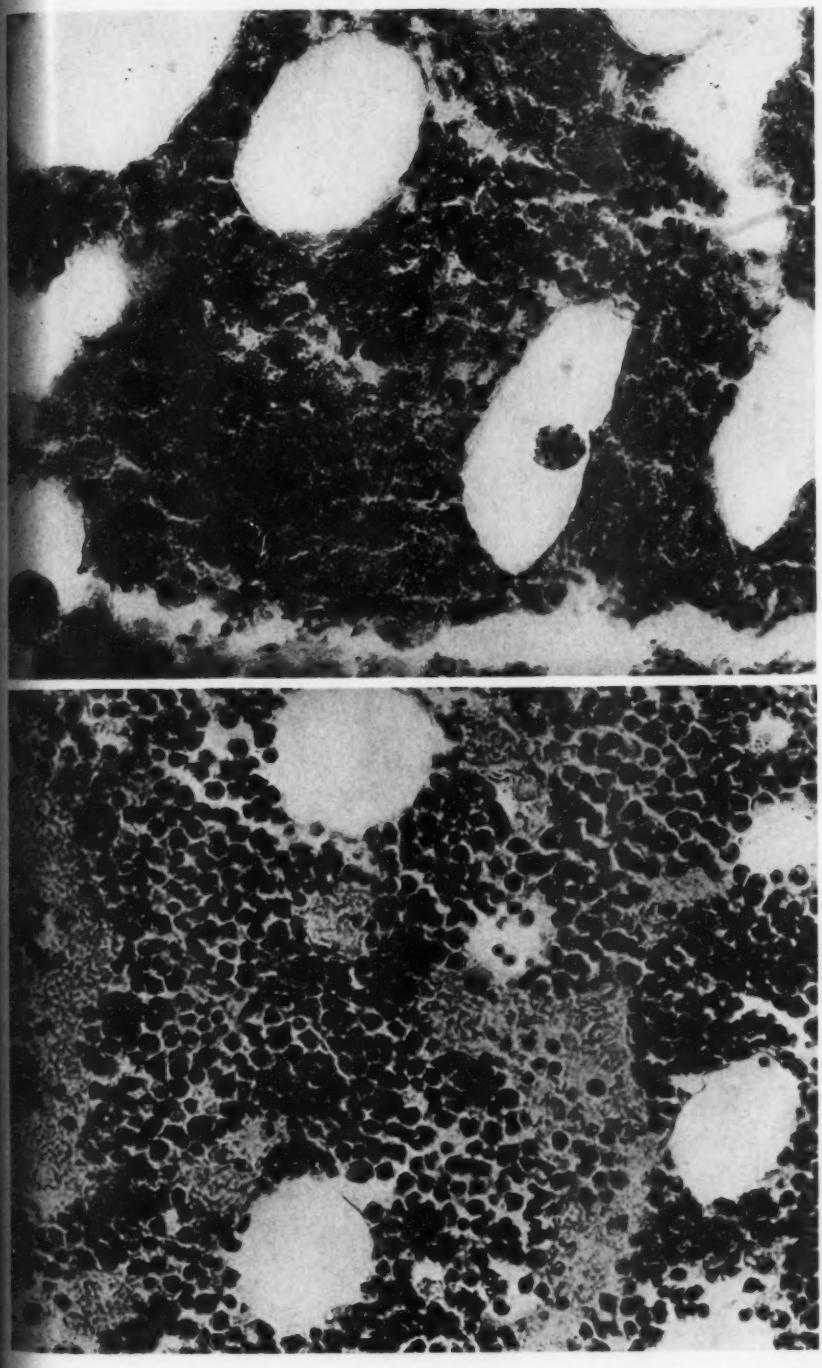
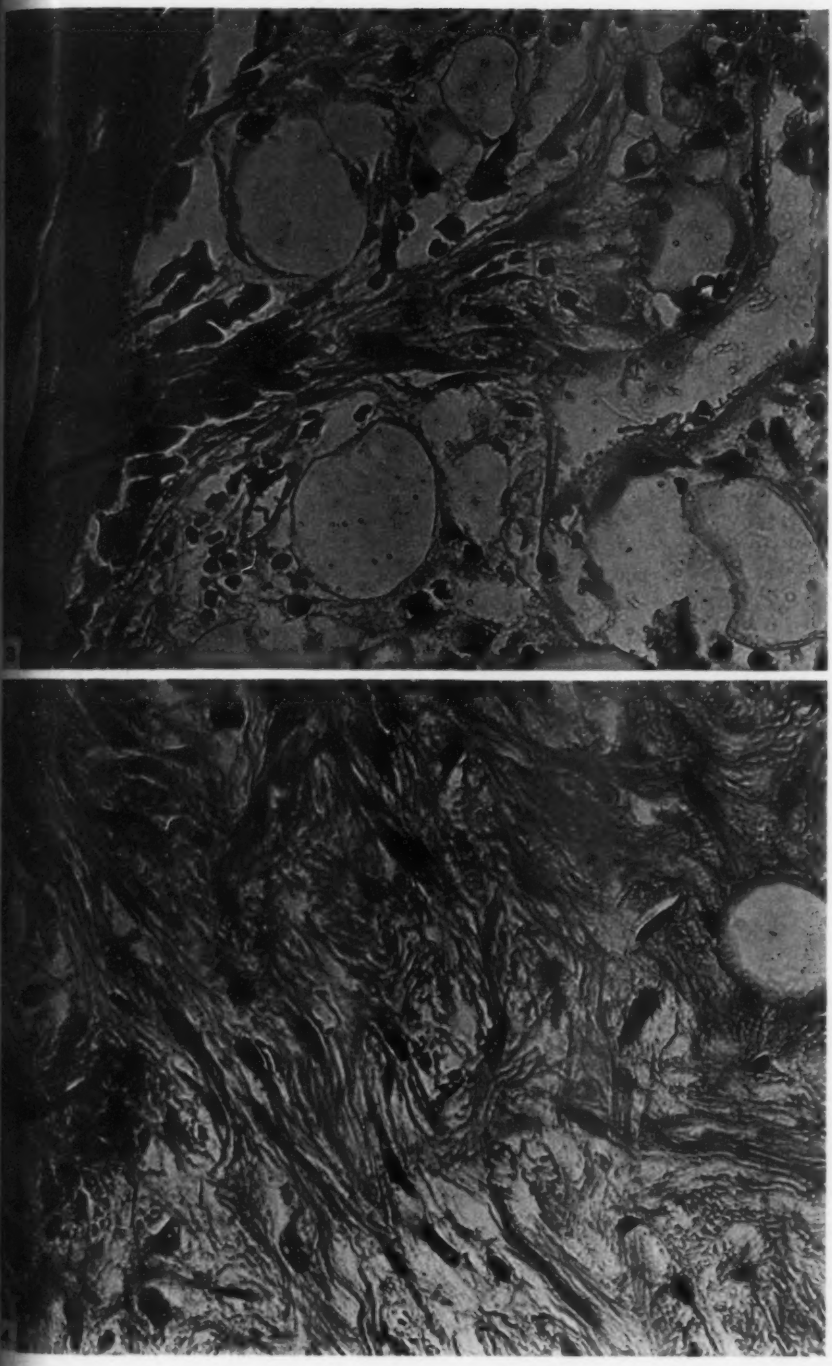


PLATE 107

FIG. 3. An area from the sternum of an animal which had received three antibone-marrow antibody injections. The large dark-staining cells on the left are thought to be endosteal cells. They lie next to a spicule of bone. × 540.

FIG. 4. An area of extensive, fibrous connective tissue overgrowth. × 125.



...ship Bone-Marrow Changes Produced by Antibodies

KIDNEY LESIONS IN STILLBORN AND NEWBORN INFANTS*

"CONGENITAL GLOMERULOSCLEROSIS"

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Pathological changes in the kidneys of fetuses and newborn infants have not been the subject of morphological investigation to any great degree in the past. Textbooks and reference works in pathology make little or no mention of such lesions, and the literature contains surprisingly few contributions in this field. Congenital anomalies, uric-acid infarcts, bile-pigment infarcts and tumors of the kidneys are briefly described and dismissed with a few sentences or paragraphs. However, pediatrists and urologists have studied clinically diseases of the kidneys in infants and young children to a greater extent.

In 1908, Karsner [1] described a case of congenital acute nephritis in an infant who died less than 1 hour after birth, and collected three additional cases from the literature. Three cases of neonatal nephritis were described by Conrad,[2] in 1938. Fishberg [3] mentioned having studied pathologically the kidneys of an infant, 4½ months old, who died from subacute glomerulonephritis. Lesions of pyelonephritis have also been described in the newborn by Craig,[4] Helmholz,[5] Hunt [6] and others.

The lesion which constitutes the subject matter of this communication was first described in 1909 by Herxheimer [7] (cited by Gruber [8]). According to him, destroyed, hyalinized glomeruli are found not infrequently in the kidneys of newborns, nurslings and also of older children as the result of developmental defects. His description can hardly be improved upon today. A free translation is as follows:

Hyalinized glomeruli are found in all layers of the cortex but most often near the surface. Sometimes they are grouped about the divisions of an interlobular artery. He found them thirty-eight times in 43 children and felt that further study might also have revealed their presence in the 5 negative cases. The incidence of the lesion did not parallel the severity of the pathological alterations. The earliest change noted by him was thickening of the parietal layer of Bowman's capsule, the result of deposition of fibrous connective tissue lamellae. Hyaline change may not be present at first. When it appears it can be seen, by means of the van Gieson stain, as a red ring of tissue about the membrana propria of Bowman's capsule. Frequently, however, the hyaline material is present only in the form of solitary foci in the

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periphery of the tuft. In these instances, he felt that serial sections might have revealed a continuity between the capsule and the tuft. In mild cases, a cleft can be seen between the glomerulus and the hyalinized capsule, the capsular epithelium having been destroyed. Further solution of the affected portion of the glomerulus may take place with gradual hyaline change; or only a sharply circumscribed area of the tuft may be affected, in which case the capsular epithelium may appear distinctly hyperplastic. Adhesions between the capsule and glomerulus are frequently noted. In some glomeruli the hyalinization appears in a more or less ball or pear-shaped form. Completely hyalinized glomeruli are also seen, spindle-shaped cells indicating the capsular boundaries. Should the hyalinization be incomplete, the unaffected portions of the glomeruli may be normal or compressed. Since the capsular portions of the tufts are often lined by rather tall epithelial cells with unusually deeply-staining nuclei, Herxheimer [7] concluded that the loops were retarded in development. Evidence of inflammatory disease was not found in the vicinity of the lesions.

Herxheimer [7] explained the basis of the eventual hyaline formation in the glomerulus by a disturbance in the synchronous development of the epithelial and mesenchymal portions of the malpighian corpuscle. The primary steps are obscure, as it is not known whether the disturbance in the stromal portion antecedes or follows the epithelial irregularity. The former was considered more likely. He saw these structures as "Hamartien" * in the sense of Albrecht, which disintegrate in the subsequent development of the kidney and then disappear.

In 1928, Schwarz [9] studied the kidneys of 80 newborn infants. There was a widespread pneumonic process in 22 infants and in 19 of these, inflammatory and infiltrative changes in the kidneys, in the form of an interstitial nephritis, were found. Lesions of the glomeruli, similar to those described by Herxheimer,[7] were found in 45 of 80 cases, an incidence of 56 per cent. However, in the cases in which inflammatory and infiltrative changes in the kidneys were present, the aforementioned glomerular changes were invariably demonstrable. Before the age of 3 weeks, however, he found the glomerular lesions in barely 30 per cent of his cases. He concluded that the changes were due to the excretion of toxic substances by the kidneys rather than to congenital anomalies, as suggested by Herxheimer.

Materials and Methods

We first noted the lesion as an incidental finding in the course of routine microscopical examinations of the kidneys of 13 stillborn and newborn infants. Some of these lesions had been previously considered

* Hamartien: defects in tissue combination during development (authors' note).

possible manifestations of a subacute glomerulonephritis. Recently the kidneys from 100 consecutive stillborn, newborn and other infants under 14 months of age were carefully examined for these lesions and 17 more examples were found. Blocks of tissue were fixed in a 4 per cent solution of formaldehyde. Hematoxylin and eosin, Mallory, McGregor and Weigert preparations were made.

Histology of the Glomerulus in the Neonatal Period

Before proceeding with a description of the lesions, review of the histogenesis of the glomerulus in early postnatal life is essential. Gruenwald and Popper [10] recently investigated this subject. The description appended below is taken from their paper:

> The glomerulus before birth consists of "an undivided globule covered by a uniform layer of high columnar epithelium which does not extend between the loops. The lumen of the glomerular loops is partly visible and contains a few erythrocytes. . . . The loops are in close contact with each other and no free spaces are left between them. At the vascular pole of the corpuscle the reflection of the visceral to the parietal layer is clearly visible.
>
> "After birth the glomerular loops expand and contain more red cells indicating an increased blood flow. Between the individual loops, clefts are visible which proceed toward the vascular pole, separating 3 to 8 lobules from each other. Parallel to this process changes in the epithelial covering take place. The originally continuous layer of high columnar epithelium is broken up by the clefts. Within the clefts the surface of the glomerular loops is partly covered by epithelium; in other parts no distinct epithelial covering can be seen. The further development leads to a stage in which the almost completely expanded loops show small islands of epithelium on most of their surface. Only on the peak is a continuous layer of high epithelium visible as a remnant of the original visceral layer. Many histological pictures suggest that a part of this high epithelium is cast off during this period, gradually losing its connection with the glomerular loops. This may account for the albuminuria during the first days of postnatal life which is indicated by clotted material in Bowman's space and the tubular lumen. . . . All these changes start in embryonic life in a greater or smaller number of glomeruli. After birth the number of maturing glomeruli increases considerably and the expansion of the capillary loops proceeds rapidly. . . .
>
> "This development is completed within the second year. . . .
>
> "The reabsorptive parts, as Henle's loops and the medullary reabsorptive capillary tufts, are fully developed at the time of birth."

The Lesion

The lesion is found indiscriminately in both kidneys. Grossly the kidneys show no abnormalities and are of average size, shape and weight. Microscopically, the lesion is distributed focally in the cortex of the kidney and is seen most often in the juxta-medullary zone.

In the 13 cases which constituted the original study, the lesion was sufficiently widespread to warrant a tentative diagnosis of "subacute glomerulonephritis" although it was recognized that the process was not a diffuse one. In the control series of 100 consecutive kidneys, 17 showed similar lesions, although not to the same extent. This might

be explained by the fact that an insufficient number of blocks were taken from these kidneys.

The lesion involves essentially the arterioles and the appertaining glomeruli. In the earliest stages there is a proliferation of the endothelium of the arterioles and of the smooth muscle cells of the media (Fig. 1). Concurrently there is an increase in the number of endothelial cells in the tuft (Fig. 2). Their nuclei are large, round or oval, and vesicular; a few are deeply stained. The epithelial cells apparently do not partake in the process at this time and their number remains unchanged. As a result of endothelial proliferation, the lumina of the capillary loops become markedly narrowed or even obliterated. The entire glomerulus becomes ischemic and only occasionally are red blood cells seen within the tufts. At the same time or shortly thereafter, but in some instances preceding the aforementioned alterations, there appears a proliferation of the parietal layer of Bowman's capsule (Figs. 3, 4 and 5). Concentric laminae of spindle-shaped cells with delicate, branching, pink-staining cytoplasm and narrow, elongated and vesicular nuclei appear. These layers, as a rule, are two to five cells in thickness and are rather loosely arranged. It is to be noted that sometimes only the tuft may partake in the process, the parietal layer of Bowman's capsule undergoing no changes. In some instances, the reverse appears to be the case. Not infrequently there is fusion of the tuft with the proliferated capsule, resulting in the formation of "crescents" (Figs. 2 and 3). During this process the walls of the vasa afferentia and arterioles have become progressively thickened and their lumina have become narrowed and even obliterated. Ischemia of the glomerulus is the natural result of such a process. Following this, homogeneous pink-staining material, staining deep blue with the Mallory and McGregor stains, appears within the tuft, or capsule, or both (Figs. 4 to 8). It is apparently deposited between the capillary loops. The deposition of this material may begin peripherally and proceed centrally or vice versa. Only a sector of the glomerulus may be involved and the remainder show no alterations. Concomitantly the number of nuclei of endothelial cells within the corpuscle diminishes until only a few remain. Within the capsule the same process takes place. Ultimately the tuft and capsule may fuse with the formation of a hyaline sphere (Fig. 8). Such occurrences are infrequent, although it is not unusual to see the glomerular tuft represented by a hyaline globule upon which is mounted a single layer of low cuboidal epithelium, separated by a broad or narrow space from Bowman's capsule. The lesion was most frequently seen in the stages of partial hyalinization of the tuft and capsule. Rarely, a few round cells and plasma cells are seen in the interstitial tissue adjoining the affected glomeruli. In

some arterioles no changes were demonstrable, while the glomeruli were the site of the alterations described; especially when the number of lesions was extremely scant and only a few were discovered in a whole section. This may be due to the fact that the vasa afferentia supplying the involved glomerulus did not appear in the section. It is also possible that these arterioles had recovered completely.

These lesions were rarely found in infants above 18 months of age. The altered glomeruli were probably completely hyalinized, leaving a delicate scar which eventually cannot be recognized.

Clinical Features

Age. The average age of the infants whose kidneys were studied was 2.7 months; 78 were less than 3 weeks of age and 22 were more. There were 4 stillborn infants. The oldest child was 14 months of age. The lesion was observed in premature infants as well as in those born at term and in first-born as well as subsequent offspring.

Sex. Among the 30 infants showing the lesion there were 22 males and 8 females, a preponderance of the former in a ratio of 2.7 to 1.

Race. Both white and colored children were affected. The greater number of white babies (24 of 30) is proportionate to the greater number of admissions of white patients.

Symptoms and Signs. There were no characteristic symptoms or signs, nor could any definite clinical syndrome be established. One patient showed anasarca, hydrothorax and ascites, which could be ascribed to renal damage; another showed only edema of the extremities. One infant died in uremia. Other symptoms and signs were referable to accompanying illnesses.

Urinalysis. In ten cases urinalysis was not performed. In the others the results of urinalysis were essentially negative, except for two cases in which occasional red blood cells were noted in the urinary sediment. Five infants showed mild albuminuria, attributable to other causes, such as fever.

Etiology and Associated Pathological Findings

The factors which theoretically may contribute to production of the lesion may be maternal or fetal, or a combination of these.

Concerning maternal factors, nothing definite could be established. There was no evidence or record of renal disease, or toxemia of pregnancy in any of the mothers. There was no high incidence of maternal infections. It is true that some of the mothers had colds during pregnancy, but whether this plays any significant rôle could not be ascertained. Five of the mothers had syphilis; active, latent, or cured. In only two instances did the children born of these mothers show positive serological tests and in only one of these were evidences of congenital

syphilis found at necropsy. The other child died from hemorrhagic encephalitis following therapy with arsenical derivatives. It is theoretically possible that some maternal factors—toxic, infectious, or hormonal—acting singly or together on the fetus *in utero*, may be responsible for the lesion described. That some of these lesions develop *in utero* cannot be questioned inasmuch as they were observed in stillborn infants. It is possible that some of the lesions of older children may have developed after birth.

As to the child itself, the lesions may be developmental, inflammatory, degenerative or vascular in origin. According to Herxheimer,[7] the frequency of the condition, its occurrence in newborn infants and the absence of evidence of any inflammatory reaction indicated to him that it was probably non-inflammatory in origin. He attributed the condition to retarded development of the kidney. Although Herxheimer made no mention of vascular changes, they were conclusively demonstrated in almost all sections studied by us. Our impression is that alterations in the blood vessels are the significant etiological factors in this condition. What causes these vascular changes is still a problem.

The associated pathological findings may be classified as follows (there is some overlapping, more than one condition having been disclosed at necropsy in many of the infants):

I. Infectious diseases
  - Meningitis, 3
  - Abscesses of skin, 2
  - Sepsis, 2
  - Peritonitis, 1
  - Otitis media, 3
  - Pneumonia, 8
  - Empyema, 1
  - Acute bacterial endocarditis, 1

II. Anomalies of the heart and great vessels, 5

III. Cerebral lesions
  - Porencephaly, 1
  - Anencephaly, 1
  - Hydrocephalus, 1
  - Hemorrhagic encephalitis, 1
  - Laceration of tentorium cerebelli with hemorrhage, 1

IV. Blood dyscrasias
  - Thrombocytopenia, 1
  - Leukemic myelosis, 1
  - Unexplained widespread hemorrhage, 1
  - Erythroblastosis, 1

V. Anomaly of kidney, 1

VI. Metabolic disorders
  - Rickets, 2

VII. Obstetrical abnormalities
  - Prematurity, 2
  - Breech presentation, 1
  - Difficult labor, 1
  - Premature separation of placenta, 1

From a consideration of these findings it is quite evident that nothing definite as to the etiology can be concluded. The lesions noted are commonly found as causes of death in infants and there is no preponderance of any single factor.

Comment

The significance of the pathological changes in the arterioles and glomeruli described above remains to be determined. They appear to be of no moment where only scattered glomeruli and arterioles are involved. Certainly their presence in 17 of 100 consecutive cases places them almost within the realm of the normal. However, when sufficiently widespread, renal functional damage may occur with the appearance of anasarca or uremia. Whether these lesions play any rôle in predisposing or leading to renal lesions in later life, such as glomerulonephritis or arterial and arteriolar sclerosis, is, of course, only conjectural.

We hesitate to apply any name to the pathological picture described. Until the etiology is ascertained, such a step would be premature. Since the lesions are both proliferative and degenerative in nature, it might be worth while, *pro tempore*, to apply the descriptive term of "congenital glomerulosclerosis" to these lesions. Their focal character possibly may be explained by irregularities in the distribution of functioning glomeruli. This is based upon the belief of some authorities that certain groups of glomeruli or even portions of a single glomerulus function at given periods of time.

These lesions were found by Herxheimer [7] in 88 per cent of the kidneys studied and by Schwarz [9] in 56 per cent. We found them in 30 of 113 cases. This low figure can be ascribed to the small number of sections studied from each case; in some only single sections of each kidney were studied.

Schwarz's [9] observation that the condition is more frequent above the age of 3 weeks has been confirmed. The incidence in infants below this age was 12.8 per cent and above this age, 31.8 per cent. We have been unable to explain this difference in incidence.

In studies upon the renal circulations, Huber,[11] Lee-Brown,[12] Loomis [13] and MacCallum [14] have described atrophic, pathologically altered glomeruli in otherwise normal kidneys (the number increasing with age) and in association with non-glomerulus-bearing blood vessels. It is believed that these vascular and glomerular changes represent involution of renal elements associated with the aging process. These lesions, we conclude, bear no relation to the condition described in this paper.

Summary

1. Certain glomerular and vascular changes in the kidneys of stillborn and newborn infants are discussed.

2. The lesions involve the arterioles and their appertaining glomeruli and are associated with hyaline changes in the tufts and capsules. They are bilateral and focal in distribution and are recognizable only by microscopical examination.

3. They are not associated with any clinical syndrome nor is their etiology or significance understood, although they are congenital and may be of vascular origin.

4. It is suggested that this lesion be termed congenital glomerulosclerosis.

NOTE: The authors acknowledge with thanks valuable suggestions in the preparation of this paper from Dr. Jean Oliver, Professor of Pathology, Long Island College of Medicine.

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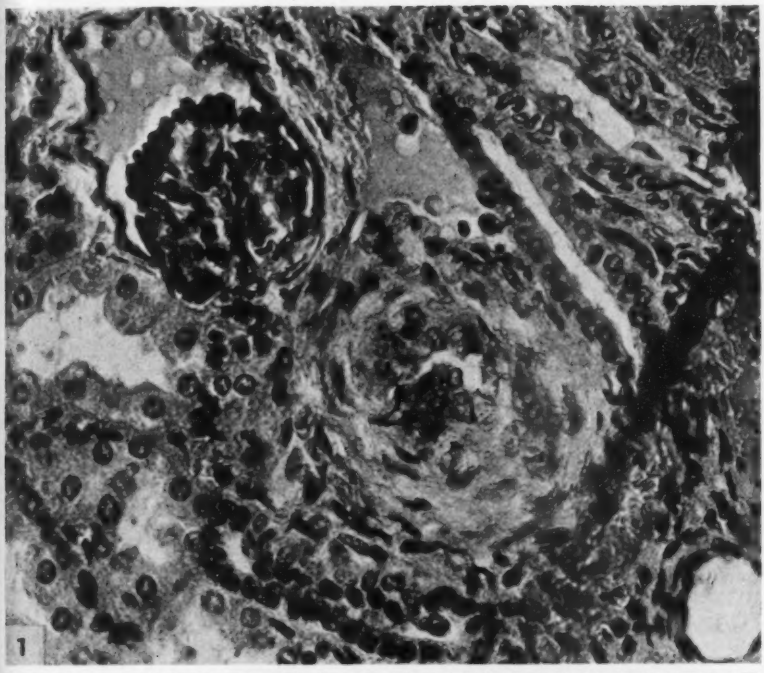
DESCRIPTION OF PLATES

PLATE 108

FIG. 1. Infant, aged 2 months. Arteriole with thickened wall, hyperplasia of endothelium and narrowing of lumen. Hematoxylin and eosin stain. × 600.

FIG. 2. Infant, aged 4 months. Endothelial and epithelial proliferation with "crescent" formation. Hematoxylin and eosin stain. × 350.


1

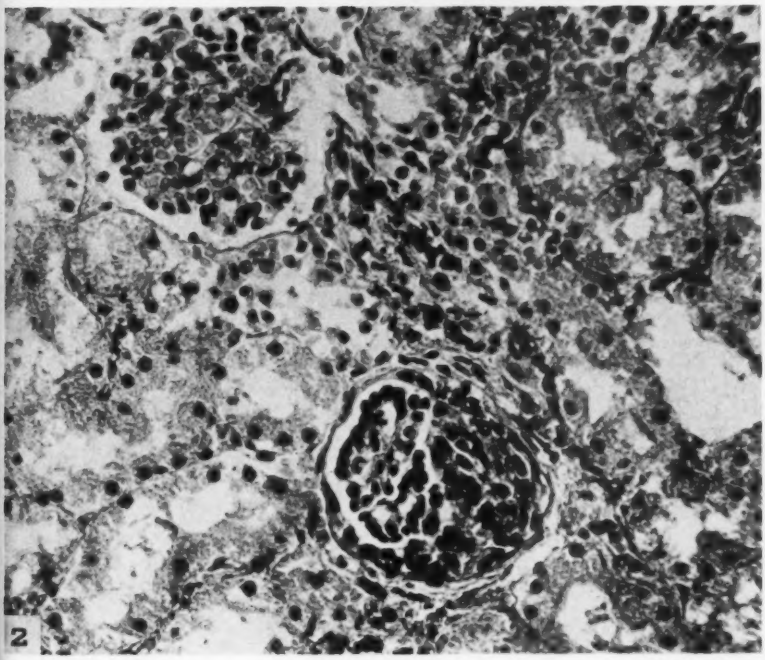
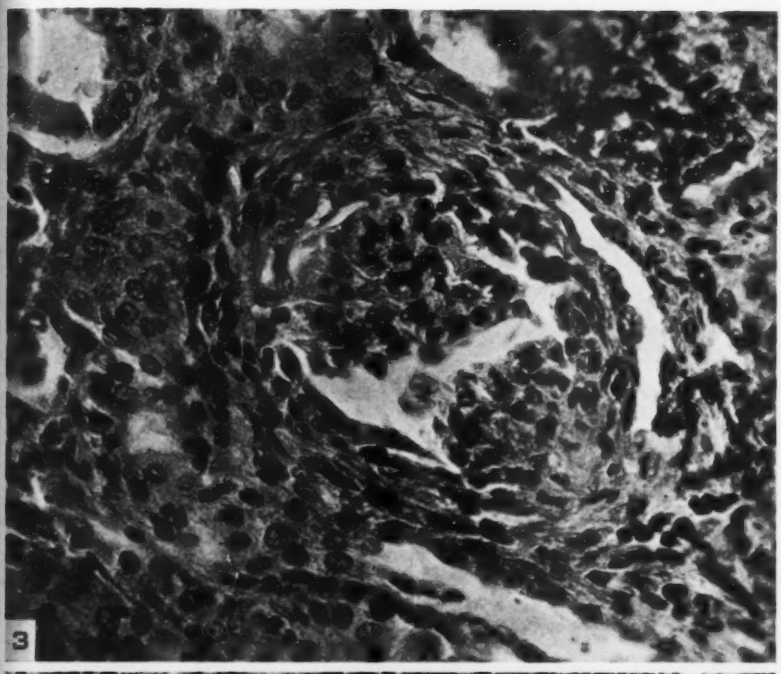

2

PLATE 109

FIG. 3. Infant, aged 2½ months. Later stage than that of Figure 2 with marked proliferative changes, fusion of tuft and capsule with almost complete obliteration of subcapsular space. Hematoxylin and eosin stain. × 900.

FIG. 4. Stillborn infant. Beginning hyaline formation with slight capsular proliferation. Hematoxylin and eosin stain. × 525.



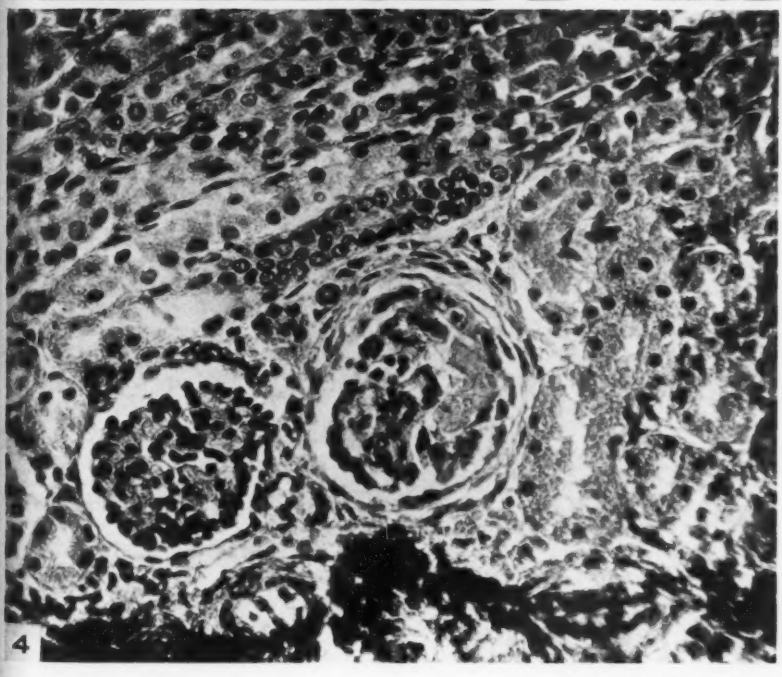


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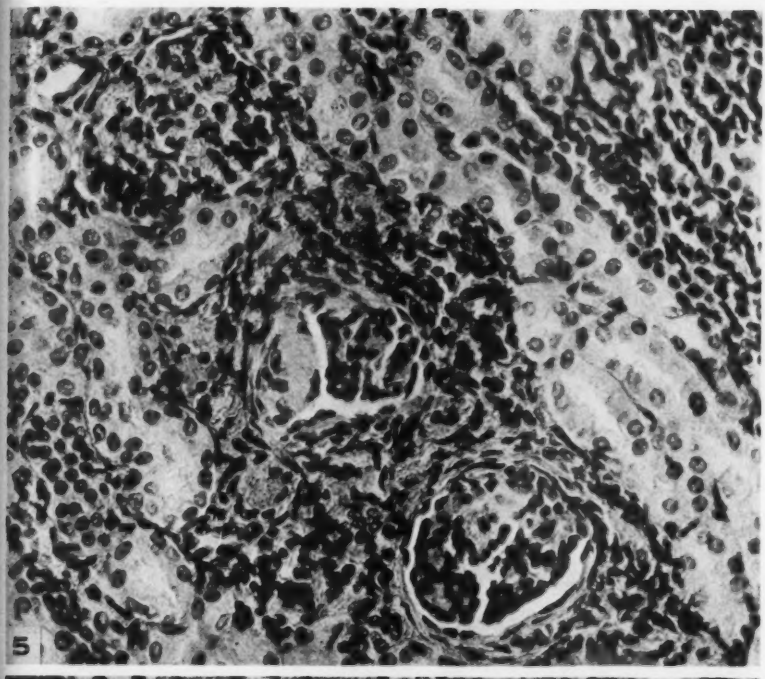
PLATE 110

FIG. 5. Infant, aged 3 weeks. Fusion of tuft and capsule, early hyaline changes and intraglomerular clefts. Hematoxylin and eosin stain. × 525.

FIG. 6. Infant, aged 12 months. Fusion of tuft and capsule, early hyaline changes and intraglomerular clefts. Hematoxylin and eosin stain. × 525.



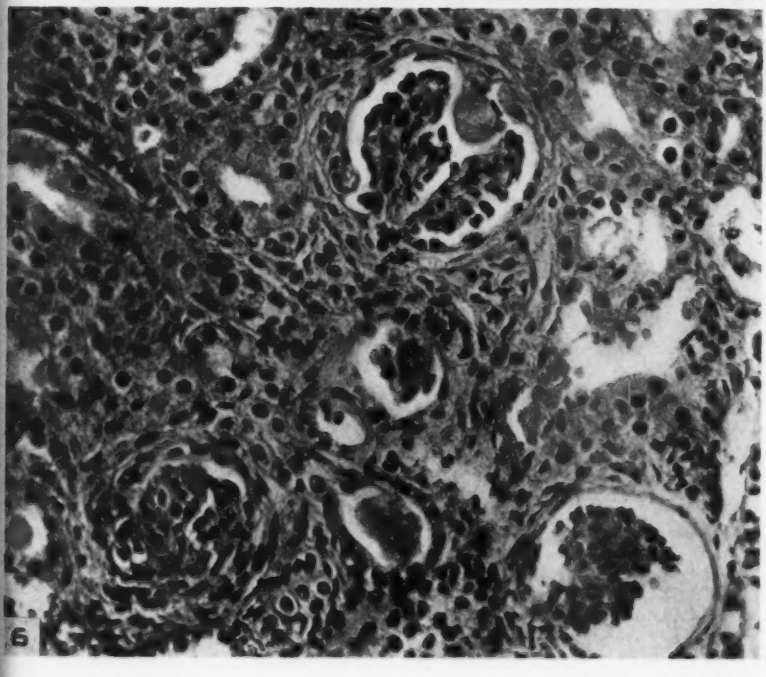


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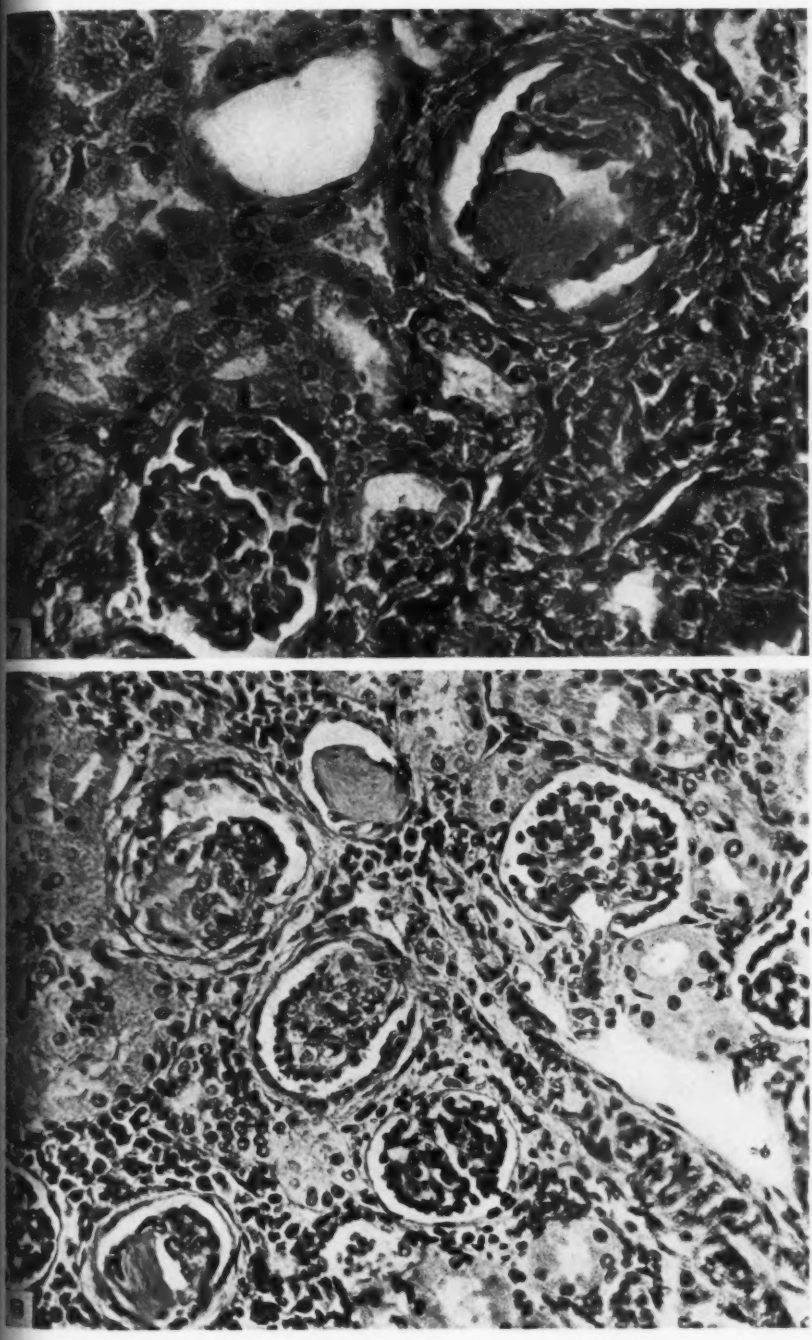
Plate III

Fig. 7. Infant, aged 6 months. Advanced hyaline changes; thickening of parietal layer of Bowman's capsule. Hematoxylin and eosin stain. × 575.

Fig. 8. Infant, aged 10 days. Late lesion with partial to complete replacement of tuft by hyaline material. Hematoxylin and eosin stain. × 440.



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CHLOROLEUKEMIA *

Report of a Case with Special Reference to Its Neoplastic Nature

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That chloroma or chloroleukemia is a rare disease is sufficiently proved by the small number of cases published since the first report of Burns [1] in 1823. Kandel [2] estimated that up till 1936, 175 undubitable cases had been reported. We have found 15 additional cases in the literature available to us: the cases of Nicod,[3] Marten and Meyer,[4] Apitz [5] (2 cases), Dustin and Thomas [6] and Jones [7] in the original and those of Laurie,[8] Zanela,[9] Roehm, Riker and Olsen,[10] Horsfall,[11] Barsoum,[12] Zemplén,[13] Frost [14] and Conti [15] in abstracts. (The case of Foulon and Desclaux [16] cannot be considered as chloroma, the green color not having been observed.)

It is now generally agreed that chloroma is a variant of myelogenous leukemia (Forkner[17] (*loc. cit.*, p. 164), Kandel[2]) in which the leukemic cells show a more marked invasiveness than in the ordinary forms of myelogenous leukemia and a stronger tendency to the formation of tumors, the color of which has given the disease its name. It is quite obvious that these qualities have contributed much to the conception that chloroma and the leukemias are neoplastic in nature. This opinion, already expressed by several authors, has recently received much emphasis from animal experiments and from observations on human leukemia, especially those of Apitz.[5, 18] The number of cases of chloroleukemia and myelogenous leukemia, published with this problem in view, *i.e.*, the relationship of leukemia to the neoplastic diseases, is still small, and so we feel justified in reporting a case of chloroleukemia in which the invasive character of the leukemic cells was very pronounced.

Report of Case

HISTORY

A colored male, native of Trinidad (B. W. I.) and 41 years old, was sent to the hospital by his physician with the following history: The patient was seen by him for the first time 8 months previously. He then complained of a swelling on the upper part of the sternum, which had grown slowly. The tumor had the size of a small fist, was moderately firm and adhered to the bone and overlying skin. As the Wassermann and Kahn reactions were strongly positive, a diagnosis of gumma was made and antisyphilitic therapy instituted. After being treated for 1 month, the patient stated that he felt very well and did not want further treatment. He was

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not seen again till the day of admission. He then appeared desperately ill, was very anemic, and voided bloody urine. His skin showed many hemorrhages. The tumor had grown somewhat in size.

The patient died a short time after admission.

REPORT OF AUTOPSY

Gross Examination

Autopsy was performed 20 minutes after death. The most important findings were as follows: There was general anemia and in the skin many hemorrhages, sometimes 2 cm. in diameter, were found. The peripheral lymph nodes were only slightly enlarged. On inspection and palpation the head showed nothing abnormal. The tumor on the manubrium sterni had the size of a man's fist, was moderately firm, adhered to the skin and the bone and infiltrated the pectoral muscles, especially on the right side. The center of the tumor was necrotic. The color of the neoplastic tissue was olive-green. In the periosteum of several ribs green tumor-tissue, which infiltrated the intercostal muscles, was found. The sternal bone was partly eroded by the tumor; the sternal marrow was also green. A large green tumor was found in the upper mediastinum; the tumor was attached to the manubrium sterni and infiltrated the upper lobes of both lungs. The pleural lymph vessels on both sides were very conspicuous and offered the same picture as in lymphangitis carcinomatosa, the only difference being that their color was green. The hilus nodes were swollen and greenish gray on section.

In the pericardium a few small, green nodules were observed; the heart was normal, except for a few petechiae in the epicardium. In the pleura diaphragmatica and in the peritoneal covering of the abdominal wall small, flat, green tumors were seen.

The spleen was enlarged, weighing 350 gm., and normal in consistency. On section the color was dark red, the trabeculae were small and the malpighian corpuscles not visible. The liver was enlarged, weighing 1900 gm. On section its color was pale red-brown. In the body of the pancreas a green tumor, measuring 1 by 3 cm., was found; the peripancreatic lymph nodes were swollen. A green tumor was situated in the cortex of the left kidney; the tumor extended into the medulla; the left renal pelvis was filled with blood clots. The marrow of the vertebrae was pale gray-red. Examination of the skull and of the brain was not possible.

Now, after preservation in formaldehyde solution for 2 years, the green color of the tumors, although somewhat faded, is still visible.

Microscopical Examination

The tissues were fixed in Heidenhain's corrosive sublimate-formaldehyde mixture (saturated aqueous solution of sublimate, two parts; water, two parts; 35 per cent formaldehyde solution, one part) and in a 7 per cent aqueous solution of formaldehyde. They were embedded in tissuemat after treatment with methylbenzoate-celloidin.

Sections were stained with hematoxylin and Giemsa's stain; orange, eosin and toluidin blue; iron hematoxylin and picrothiazine; hematoxylin and orcein; azocarmine and aniline blue, and Masson's tetrachrome stain according to the modification of Larson and Levin.[10] For the peroxidase reaction the method of Sato was used on frozen sections.

General Description of the Leukemic Cells. The leukemic cells had a size two or three times that of an erythrocyte; the shape was round, oval or more irregular, due to reciprocal pressure and adaptation to the available space; the cells were in general distinctly outlined. The nuclei were relatively large with a sharp nuclear membrane and stained lightly; they were in general round or oval, but in many cells more or less deeply indented, sometimes on both sides. The nuclei contained small chromatin granules and always one or two conspicuous nucleoli. The protoplasm was basophilic, although not so strongly as that of plasma cells or as the basal part of the acinar cells of the pancreas, in which organ this difference in staining reaction can be easily observed. In sections stained with hematoxylin and Giemsa's stain sometimes very small, pale red rods or granules or a small pink-colored area were found in the protoplasm. Basophil, eosinophil or neutrophil granules were entirely absent. The peroxidase reaction was negative. Mitotic divisions were numerous.

Pectoral and Intercostal Muscles. In the pectoral and intercostal muscles the invasive properties of the leukemic cells could be most easily observed. The muscle fibers were separated by large collections of leukemic cells and were often atrophic. In other places they were still in more or less close contact and were apparently normal. Many fibers were invaded by the leukemic cells, and in such a way that the peripheral sarcoplasm, with its nuclei and fibers, formed a hollow and sometimes thick-walled cylinder, in the lumen of which the leukemic cells were found, and, as proved by the presence of mitotic divisions, were proliferating. Sometimes the cells were so closely packed that the cell borders were indistinct. The fibers in the walls of the cylinders were easily demonstrated in cross and longitudinal sections and in the latter the cross striations were clearly visible, especially when the Masson stain was used. On longitudinal sections, sometimes thin strands

of protoplasm, recognizable in Masson-stained sections by their red color, could be seen running through the masses of leukemic cells from one side of the cylinder to the other, so that the lumen seemed to be divided into small compartments. As this could be observed also in cross sections of the muscle fibers, it cannot be due to tangential sectioning.

When the number of leukemic cells increased, the walls of the cylinders became thinner and only a very thin layer of sarcoplasm remained, in which, however, the fibrils persisted in many cases. The Masson stain was again the most useful for the demonstration of these last remnants of the muscle fibers. The next and last stage was the complete disappearance of the muscle fibers. In several places, where the muscular tissue had been completely replaced by leukemic cells, intact muscle spindles were found.

Lungs. The pleura of the upper lobes was much thickened and the leukemic cells were found lying between thick bundles of collagen, from which finer bundles and thin fibers were branching off. Often the leukemic cells were surrounded by very fine fibers, which stained red with picrothiazine. The lymph vessels of the pleura were distended with closely packed leukemic cells. In the lungs, infiltration was very heavy in the interlobular septa, which were greatly thickened, and around the bronchi and greater blood vessels, which were surrounded by enormous masses of leukemic cells. Many mitotic divisions were found here. The walls of the bronchi were invaded in many places and the leukemic cells were lying just below the bronchial epithelium. This epithelium had completely disappeared in some places and here the leukemic cells were found in the bronchial lumen. Invasion of the walls of the blood vessels was also observed, the leukemic cells proliferating between the media and the endothelium, which was pushed towards the lumen. Sometimes the smaller blood vessels were filled with leukemic cells, some of which were dividing, so that these vessels could not be distinguished from vessels filled with tumor thrombi. Dividing leukemic cells were also found in vessels containing but a few cells. Where the intrapulmonary lymphatics were still recognizable, they were filled with leukemic cells. The alveolar septa were thickened by infiltration with leukemic cells, which were also found within the capillaries. The "alveolar cells" were swollen and in the alveoli huge collections of leukemic cells, together with large vacuolated, often dust-loaded macrophages, were seen. In the alveolar lumina the leukemic cells were proliferating, as proved again by the presence of mitoses. Polymorphonuclear leukocytes were entirely absent.

Spleen. The structure of the spleen had remained intact as was

shown by the connective tissue stains; the walls of the venous sinuses were normal. The malpighian corpuscles were small. In the sinuses and in the pulpa reticulum many leukemic cells were found. The cells were not closely packed; mitotic divisions were frequent. In some sinuses large macrophages, loaded with hemosiderin, were seen. Only after prolonged searching could a few polymorphonuclear leukocytes be found.

Liver. The capillaries and the periportal connective tissue contained only a few leukemic cells, some of which were dividing. Many "triangles" were entirely free from leukemic cells.

Pancreas. Sections taken from the border of the green tumor showed important changes. The pancreatic tissue was densely infiltrated by leukemic cells and the acini were atrophic or had completely disappeared. In the atrophic acini the acinar cells still contained zymogen granules. Many islands were still intact and very conspicuous, and their different cell types were easily recognizable. In some places the invasion of islands by leukemic cells could be observed. In general the leukemic cells were lying in the meshes of an amazingly rich network of thin, branching fibers and were actively proliferating. They also infiltrated the neighboring fat tissue.

Kidney. Over the green tumor the capsule was thickened and contained many leukemic cells. In structure the capsule resembled the infiltrated pleura. The cortex was heavily infiltrated; the renal parenchyma had almost completely disappeared, only a few glomeruli and atrophic tubules remaining. The leukemic cells were closely packed and showed many mitotic divisions, which, however, were not evenly distributed.

Heart. In the epicardial fat and between the muscle fibers the leukemic cells were present in small collections; on the contrary, enormous masses of leukemic cells were found in the ventricles caught between the trabeculae.

Lymph Nodes. The peripancreatic lymph nodes were invaded from without, the marginal sinus being full of leukemic cells which from there penetrated the tissue of the gland. In the hilus nodes of the lungs the lymphatic tissue was almost completely replaced by leukemic cells. True myeloid metaplasia could not be observed.

Bone Marrow. The vertebral marrow was very cellular; however, only relatively few normal cells were found, mostly nucleated red cells and eosinophilic myelocytes and leukocytes. Neutrophilic cells and megakaryocytes were rare. The other cells, found in the marrow, were leukemic cells.

Blood Vessels. Apart from the blood vessels in the lungs, vessels

in other parts also were invaded, especially the veins in the pectoral muscles, the walls of which were destroyed by the leukemic cells, only a few smooth muscle fibers being recognizable in Masson-stained sections.

Discussion

The color of the tumors and infiltrations is in itself sufficient for the diagnosis of chloroma; however, as blood counts and the study of blood films were impossible, the evidence for the diagnosis of myelogenous leukemia must be gathered from the anatomical and histological findings. The almost complete absence of polymorphonuclear leukocytes from the different organs and from the blood; the fact that in the lumina of the blood vessels only the cells, previously described as leukemic, were found, and the extensive replacement of the bone marrow by these cells constitute, in our opinion, sufficient proof. The slight histological changes found in the liver are not so typical for ordinary leukemia, but occur often in chloroma (Dustin and Thomas,[6] Kandel,[2] Nicod [3]). We believe also that the cytological details of the leukemic cells leave little doubt as to their nature. The size of the cells, the structure of their nuclei with the fine chromatin granules and the large nucleoli are much more typical for undifferentiated myelogenous cells or myeloblasts than for lymphocytes and their parent cells. When we compare the cells found in our case with those described and depicted by Forkner [20] in monocytic leukemia, this disease can be easily excluded. We cannot attach much importance to the fact that in our case the peroxidase reaction was negative. Premature bone-marrow cells may show a negative reaction [Piney,[21] Downey,[22] Jaffé,[23] Forkner [17] (*loc. cit.*, p. 51)] and it is also possible that the long preservation of the tissues in formaldehyde solution (18 months) was responsible for this result.

As stated in the introduction, the conception of the leukemias as neoplastic in nature (Babes,[24] de Vries,[25] Lignac [26] and others) has received much support from experimental studies on leukemia in rodents (Snijders,[27] Tio Tjwan Gie,[28] Furth,[29] Hall and Knocke,[30] Murphy and Sturm [31]). However, as some authors seen to suggest that human leukemia and the transmissible leukemias of rodents are not analogous (Israëls [32]), we must turn to the analysis of human cases and here the observations of Apitz [5,18] are of special interest. In two papers he described twelve cases of human leukemia, offering much evidence for the conception mentioned above. Three of his cases were myelogenous leukemias and these showed the following features: In case no. 1 the cervical, tracheal, perigastric and retroperitoneal lymph

nodes showed on section a distinctly green color, and in the left retroperitoneal space a large, partly necrotic, yellowish white tumor was found. This tumor infiltrated the muscles and was composed of myeloblasts and myelocytes, part of the latter containing eosinophilic granules. The cells proliferated inside small venules, formed tumor thrombi and infiltrated the neighboring fat tissue. Here even megakaryocytes were seen. In case no. 2 a large hemorrhagic tumor was found between the muscles of the thigh, composed of myeloblasts and myelocytes and infiltrating the muscles. It is of special interest that in this case the leukemic cells proliferated inside empty sarcolemma tubes and the author emphasized the fact that he considered the sarcolemma as previously emptied by necrosis of the muscle fibers. In case no. 3 the paratracheal and the right cervical lymph nodes showed a greenish color and here the leukemic cells showed a special aggressiveness and infiltrated the muscles and the wall of the jugular vein.

When we compare these findings of Apitz [5,18] with the anatomical and histological data of our case, we cannot fail to observe that, although the topographical localization of the lesions is different, a very great similarity exists. We, too, found invasion and destruction of the walls of blood vessels, the formation of tumor thrombi, the destruction of the bronchial wall and of the parenchyma of the pancreas and kidney; the invasion of the muscles could be observed even better than in the second case of Apitz. We could show that the muscle fibers themselves were invaded by the leukemic cells, just as the muscle fibers of the tongue are invaded by a lingual carcinoma and the pectoral muscles by a carcinoma mammae. The changes in the pleural lymphatics were the same as in the so-called lymphangitis carcinomatosa (Kaufmann [33]), the only difference being that the lymph vessels were filled with leukemic cells and that their color was green. Finally, the rapid proliferation of the leukemic cells could be proved by the presence of numerous mitotic divisions.

Were it not for the fact that *normal* blood cells are capable of infiltrating other tissues, perhaps few investigators would doubt the blastomatous nature of such cases as they show all the features considered necessary for the diagnosis of a malignant tumor. In connection with this we first wish to point out that, although invasiveness is an inherent property of the chorionic epithelium, nobody seems to doubt the neoplastic nature of chorio-epithelioma; and, secondly, that it is not sufficiently proved that *normal* myeloblasts from non-leukemic men and animals can invade other tissues in the same way as mature leukocytes in inflammation and the *abnormal* myeloblasts in leukemia.

A much used argument against the neoplastic nature of the leukemias

is the fact that in most cases no tumors can be found. This, however, can also occur in diffuse gliomatosis of the leptomeninges (Connor and Cushing [34]), which is considered a truly neoplastic process. The opinion expressed by Kandel [2] that the myeloblasts, which are already showing a rapid and disorderly proliferation, simply assume another rôle in chloroma and become capable of destructive invasion, is in contradiction with the modern conceptions of malignant tumors.

When, finally, we compare the definition of a tumor given by Ewing,[35] "a tumor is an autonomous new growth of tissue," with Forkner's [17] (*loc. cit.*, p. 1) definition of leukemia, "an invariably fatal systemic disease of unknown etiology, primarily involving the blood-forming organs and characterized by widespread, rapid, and disorderly proliferation of the leukocytes and their precursors," we see that the most important difference lies in the expression "systemic disease." It should, however, be remembered that the conception of leukemia as a systemic disease originates from a time when far less was known about tumors and the leukemias themselves and was for many years carried on through medical, especially clinical, literature, without adequate proof being offered.

Summary

A case of chloroleukemia, occurring in a colored male, 41 years old, is described. The leukemic cells were undifferentiated myelogenous elements, which invaded muscle fibers, blood vessels, lymphatics and other tissues in the same manner as tumors generally recognized as malignant. The relationship between the leukemias and the neoplasms is discussed briefly and the similarity of the two processes emphasized.

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DESCRIPTION OF PLATES

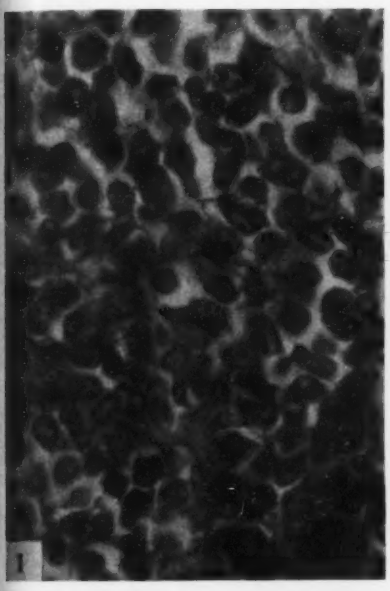
PLATE 112

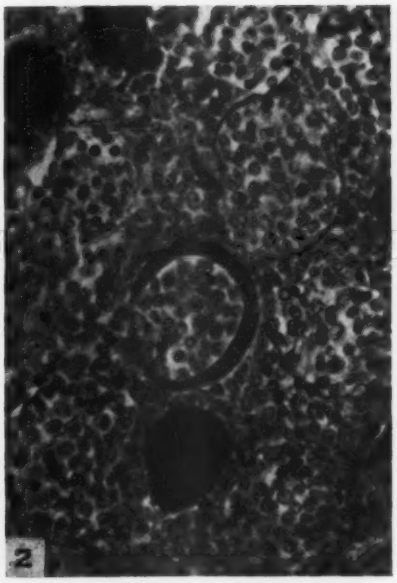
FIG. 1. Leukemic cells. × 750.

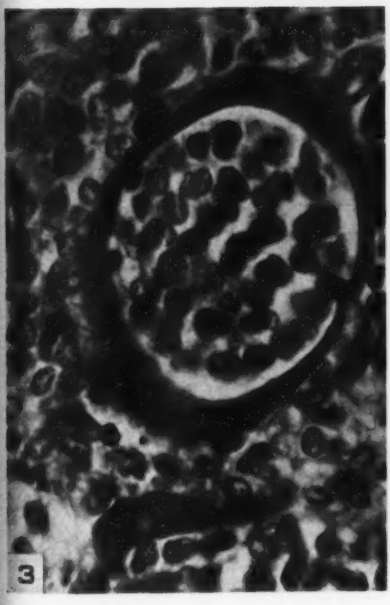
FIG. 2. Transverse section of the pectoral muscle. The leukemic cells are seen lying inside and between the muscle fibers. In one muscle fiber three mitotic divisions are seen. × 357.

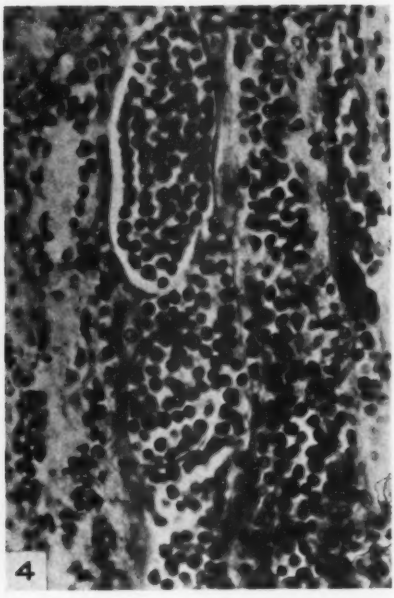
FIG. 3. From a section of the pectoral muscle. The muscle fibrils are visible. × 750.

FIG. 4. Longitudinal section of muscle fibers infiltrated by leukemic cells. The hollow cylinder seems to be divided into small compartments. × 357.









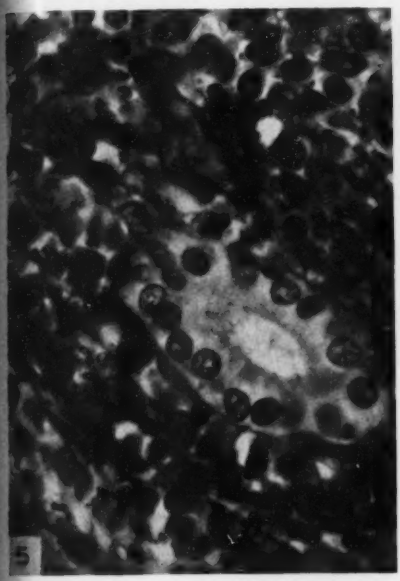
Fritz and van der Sar Chloroleukemia

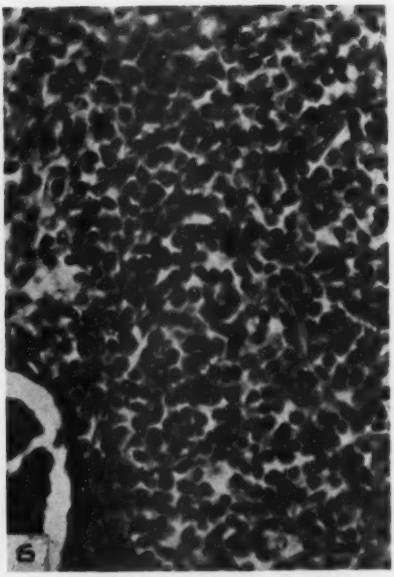
PLATE 113

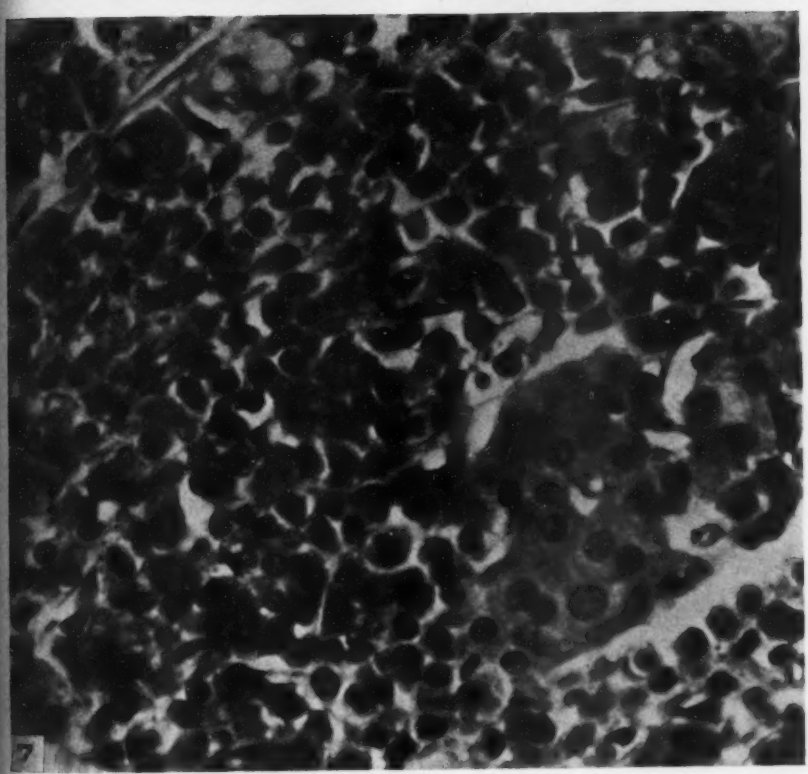
FIG. 5. Small pancreatic duct surrounded by leukemic cells. There is one mitotic figure in the field. × 750.

FIG. 6. Pancreas. The beginning infiltration of an islet is shown. × 715.

FIG. 7. Renal cortex with a heavy infiltration of leukemic cells. × 357.







THE HEART IN UREMIA *

CYRIL SOLOMON, M.D., J. EUGENE ROBERTS, M.D., and JAMES R. LISA, M.D.
(From the Pathological Laboratory, City Hospital, Welfare Island, Department of Hospitals, New York, N. Y.)

This communication is a report of the pathological findings in the hearts of 50 patients dying while in uremia. Acute endothelial hyperplasia of the coronary arterioles, producing narrowing or thrombosis, was present in patients with renal necrotizing arteriolitis. With this one exception, there were no cardiac changes characteristic either of the uremic state or of the associated renal pathology.

The selection of the material rested upon the clinical diagnosis of uremia and the histological demonstration of kidney damage compatible with complete renal decompensation. The criteria for the diagnosis of uremia were an azotemia of not less than 90 mg. of blood nonprotein nitrogen, urinary abnormalities and coma. Prerenal azotemias were eliminated from consideration. The pathological classification of the kidneys was the same as that adopted in another study.[1]

The kidneys were classified in five groups. There were 18 cases of pyelonephritis, 10 of nephrosclerosis, 8 of necrotizing arteriolitis, 4 of acute diffuse glomerulonephritis and 9 of chronic diffuse glomerulonephritis. All kidneys with necrotizing arteriolitis had a greater or less degree of nephrosclerosis.

The weights of 49 hearts were recorded. They ranged from 175 to 940 gm. One heart which was not weighed was noted as hypertrophied. The smallest heart was from a child of 9 years with chronic diffuse glomerulonephritis; the myocardium was definitely hypertrophied. As can be seen from Table I, the widest ranges were found in pyelonephritis and nephrosclerosis. The greatest weights tended to occur with renal necrotizing arteriolitis.

Acute diffuse fibrinous pericarditis was present in 7 cases. An acute fibrinous pericarditis limited to the base of the heart and the intrapericardial portions of the large vessels was present in 5 others. Chronic adhesive pericarditis, in one instance of rheumatic nature, was found in 6. Although the fibrinous pericarditis in uremia is usually considered to be a response to a chemical irritant, it is of interest to note that in 4 cases studied bacteriologically, streptococci were isolated from the pericardial exudate in each instance.

Arteriosclerosis of the superficial coronary arteries varied greatly in degree but tended to be only moderate. Frequently the vessels were

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normal or slightly affected. Twelve had severe involvement: 4 each with pyelonephritis and nephrosclerosis, 3 with chronic glomerulonephritis and 1 with necrotizing arteriolitis. There was no close relation between the degree of coronary arteriosclerosis and either the renal pathology or the weights of the hearts.

The myocardium in the majority of the hearts was pale, mottled yellow or gray, sometimes with yellow or reddish brown streaking, and was of poor consistence. There were 2 instances of fresh massive cardiac infarction: 1 showed recent thrombosis and severe coronary arteriosclerosis; the other, normal coronary arteries without demonstrable

TABLE I

Weight of Heart According to Renal Pathology

	Weight of heart in gm.				
	450 or less	451–550	551–650	651 or more	Total
Pyelonephritis	10	1	2	5	18
Nephrosclerosis	3	3	1	3	10
Necrotizing arteriolitis	0	0	7	1	8
Acute diffuse glomerulitis	2	0	1	1	4
Chronic diffuse glomerulitis	7	1	1	0	9

thrombosis either grossly or microscopically. Healed massive infarctions were present in 3, all associated with the more severe degrees of arteriosclerosis of the superficial branches. One of these hearts had an interventricular aneurysm through which a small, healed, pin-point perforation connected the ventricular cavities.

The most interesting microscopical observation was the change found in the myocardial branches of the coronary arteries in the cases of renal necrotizing arteriolitis. The arterioles in 7 instances had an extreme endothelial hyperplasia, causing partial or complete occlusion. In 1 there was necrosis of the wall, polymorphonuclear infiltration and demonstrable bacteria. It apparently was a true infectious arteritis. A mild endothelial hyperplasia was found in 1 case of pyelonephritis. Moderate or marked atherosclerosis of the larger intrinsic arteries, with focal distribution, was present in 20 hearts, usually in parallel with similar changes of the superficial branches. Marked reduplication of the internal elastica was noted in 1 instance. No changes of any significance were found in 30 hearts.

The microscopical lesions of the myocardial fibers were of many types. Fatty degeneration was the most common, being present in 49 of the 50 hearts. Gouley [2] stressed the occurrence of a very fine, fatty degeneration of the myocardium showing a marked tendency to be most intense in the subepicardial layer. He stated that it was more

prone to appear in patients with hypertension. We were unable to confirm these observations. In our series there was no tendency for the degeneration to occur in any specific region. It was found in all layers, was usually diffuse and not limited to any particular zone. No relation could be found between its presence and the type of renal pathology or the blood pressure. There was slight fatty degeneration in 10 hearts, mild in 25, moderate in 13 and marked in 1.

Miliary myocardial necroses [3] were next in order of frequency. They were present in 35 instances, rare or scanty in 24, in moderate numbers in 10 and very numerous in 1. They seldom occurred as isolated lesions but were usually associated with acute interstitial myocarditis. This association suggests that these two lesions probably have a common underlying etiology.

Acute interstitial myocarditis was present almost as frequently as the myocardial necroses. It was found in 31 hearts, slight or mild in degree in 14, moderately extensive in 14 and very marked in 3. Bacteria were found in 3.

Acute miliary infarctions [4] were less common. In 10 hearts they were scanty, in 3 in moderate numbers and in 5 very numerous. In 2 hearts bacteria were present.

There were other lesions affecting the hearts in small number. There were 5 cases of rheumatic heart disease with valvular deformities. One was associated with extensive acute rheumatic myocarditis. Three hearts gave evidence of syphilis, twice affecting the aortic valve, once limited to the aorta. An acute vegetative endocarditis of the aortic valve was found twice. Two hearts had recent mural thrombi of the chambers; hemolytic streptococci were isolated from both.

Certain relations existed between the acute myocardal lesions and the renal pathology. Seven of the 8 cases of renal necrotizing arteriolitis had extensive severe damage of all types, although the miliary infarctions were the most prominent. Since these hearts had acute arteriolar changes, the presence of the myocardial damage could reasonably be attributed to them. About half of the nephrosclerotic patients had cardiac lesions of one type or another, miliary infarction being slightly predominant. Among those with pyelonephritis, the types of lesions exhibited a different proportion. Two-thirds, or 12, had acute interstitial myocarditis; one-third, or 6, had miliary necroses. Miliary infarctions occurred in only 3, 2 of whom had severe arteriosclerosis. Cardiac lesions among those with glomerulonephritis were uncommon.

Cardiac symptoms were present with the signs of uremia throughout the period of observation in 26 patients: congestive failure in 19 and coronary or left ventricular failure in 7. In the group with congestive

failure, the renal changes were pyelonephritis in 6, nephrosclerosis in 6, necrotizing arteriolitis in 4 and glomerulitis in 3. In the coronary group, there were 2 each of pyelonephritis, nephrosclerosis and glomerulonephritis and 1 of necrotizing arteriolitis. In 25 of the 26 cases, acute myocardial lesions were present, exclusive of fatty degeneration. One case of pyelonephritis did not have demonstrable lesions.

Twenty-four cases were free of cardiac symptoms, except for terminal phenomena in a few instances. The renal lesions were pyelonephritis in 10, nephrosclerosis in 3, necrotizing arteriolitis in 3 and glomerulonephritis in 8. One case of arteriolitis had a terminal left ventricular failure of 7 hours' duration; the acute myocardial lesions were extensive and localized to the left ventricle and interventricular wall. The second case died suddenly; the lesions were very acute and concentrated on the left side. In the remaining cases, the cardiac lesions were either entirely absent or very moderate in extent.

Summary and Conclusions

The pathological findings in 50 hearts of patients dying in uremia are reported. No lesion was found which could be considered characteristic of the uremic state. An unusual endothelial hyperplasia of the cardiac arterioles was present in 7 of 8 cases with acute necrotizing arteriolitis of the kidneys. In no other type of renal pathology was there any correlation with the cardiac changes. A definite relation existed between the presence of acute lesions of the myocardial fibers and the occurrence of clinical signs of cardiac dysfunction.

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FORTY-SECOND ANNUAL MEETING
OF THE
AMERICAN ASSOCIATION OF PATHOLOGISTS
AND BACTERIOLOGISTS

ST. LOUIS

APRIL SECOND AND THIRD, 1942

THE AMERICAN ASSOCIATION OF PATHOLOGISTS AND BACTERIOLOGISTS

Forty-Second Annual Meeting, Washington University School of Medicine, St. Louis, Missouri

April Second and Third, 1942

PRESIDENT HAYTHORN IN THE CHAIR

BUSINESS MEETING

April Second, 1942

Upon nomination of the Council, the following officers were elected by the Association:

President	PAUL R. CANNON
Vice-President	WILEY D. FORBUS
Secretary	HOWARD T. KARSNER
Treasurer	ALAN R. MORITZ
Incoming Member of Council	SHIELDS WARREN
Assistant Secretary	FRANCIS BAYLESS
Assistant Treasurer	GRANVILLE A. BENNETT

The Secretary announced the election of the following new members:

Arthur Charles Allen
W. A. D. Anderson
Alfred Angrist
Chapman H. Binford
Frank Bloom
Paul Edmund Boyle
Alfred Cohn
William Dock
John H. Fisher
Frank W. Foote, Jr.
Bruno Gerstl
Hugh R. Gilmore, Jr.
Leonard J. Goss
Sylvester Emanuel Gould
Thomas R. Hamilton
Frank Lappin Horsfall, Jr.
Thomas DeArman Kinney
Karl Lowenthal
Theodore L. Perrin
Abou D. Pollack
Geoffrey William Rake
Clara Raven
Cesare Tedeschi
Henry James Tweddell
George A. Walker
Tobias Weinberg
Robert J. Williams

The Secretary reported the reinstatement to membership of:

Charles B. McGlumphy
Margaret Warwick

The Secretary announced the election of Esmond R. Long as representative of the Association in the Division of Medical Sciences of the National Research Council.

The Secretary announced the election of Jacob Furth to succeed Herbert Fox as a member of the Advisory Committee of the Lymphatic Tumor Registry.

The Secretary announced the election of Malcolm H. Soule to succeed himself in the Editorial Board of the *American Journal of Pathology*.

The Secretary announced that the next meeting of the Association will be held as the guests of the University of Chicago in Chicago, Illinois, April 1 and 2, 1943.

The Secretary announced the selection of James Ewing of New York as recipient of the Gold Headed Cane of the American Association of Pathologists and Bacteriologists.

The Secretary drew attention to the fact that at the last meeting of the Association the Council had recommended that in Article 2 of the By-Laws, the sentence "The Council shall consist of seven members elected by the Association, and the Secretary and Treasurer, ex officio," be changed to read, "The Council shall consist of seven members elected by the Association, and the Secretary, Treasurer and Editor of the *American Journal of Pathology* ex officio."

Upon motion, duly made and seconded, the above change in the By-Laws was approved by the Association.

SCIENTIFIC PROCEEDINGS

FATAL MATERNAL PULMONARY EMBOLISM BY AMNIOTIC FLUID. C. C. Lushbaugh (by invitation) and Paul E. Steiner, Chicago, Ill.

Abstract. On the basis of a study of ten women, together with experiments on animals, a new obstetric disease was recognized which has its distinctive symptomatology, etiology and pathology. The series was composed of cases which previously had been considered obstetric shock, idiopathic postpartum uterine atony with hemorrhage, acute pulmonary edema of pregnancy and as other obscure diseases, for all of which a causation had not previously been shown. Clinically the disease was characterized by shock coming on during labor or soon after its conclusion. Predisposing factors were uterine tetany or exceptionally strong uterine contractions, meconium in the amniotic fluid, intra-uterine death of the fetus, an oversize baby, multiparity and advanced age of the mother. The essential pathologic basis was found on microscopic examination of the lungs. It consisted of widespread embolism of small pulmonary arteries, arterioles and capillaries by the particulate matter found in amniotic fluid and by meconium. The disease was duplicated clinically and pathologically in rabbits and dogs by the intravenous injection of human amniotic fluid and meconium. The incidence of fatal cases was 0.2 per cent in general autopsies and 1 to 8,000 confinements. The incidence of sublethal cases is unknown, but these cases probably outnumber the fatalities. This disease was found to be the commonest cause of obstetric death during labor or in the first 10 hours of the puerperium.

AGING IN THE HUMAN PLACENTA. Louis M. Hellman and (by invitation) L. M. Flexner and A. Gellhorn, Baltimore, Md.

Abstract. The human placenta has a limited life span of approximately 280 days. During this brief period the processes of aging, normally occupying years, take place with great rapidity. Included under this heading is a great decrease in size and an increase in number of villi. The stroma becomes more compact and the fetal vessels increase in number. Degenerative changes occur in the covering coats, including the deposition of fibrin, fat and calcium. Inasmuch as the syncytium not only covers the villus but also lines the intervillous space, degenerative changes here are somewhat analogous to arterial changes associated with senility, and, similarly, thrombosis may ensue. On the positive side these old-age changes in the placenta result in greater ease of transfer from mother to fetus. This is demonstrated experimentally by a sixfold increase in the passage of radioactive sodium per gram of placenta from early pregnancy to term.

Discussion

(Dr. Carl V. Weller, Ann Arbor, Mich.) I would like to ask Dr. Hellman how sharp an end-point as to placental age can be established by the disappearance of the Langhans cells.

(Dr. Hellman) I do not think we know exactly—somewhere between the third and fourth month most of the Langhans cells disappear. Wislocki has said recently that if you examine the full-term placenta closely you can find Langhans cells.

FIBROUS PLEURAL ADHESIONS. Edward Smith (by invitation), St. Louis, Mo.

Abstract. Fibrous pleural adhesions were found in 66 per cent of 400 unselected autopsies upon patients varying from 1 to 89 years of age. The cases were studied grossly and microscopically without the aid of x-ray. The incidence of fibrous pleural adhesions increased from none at birth to 79 per cent in individuals 50

years of age. Lungs with calcified nodules and apical scars did not show a significantly increased incidence of fibrous pleural adhesions. Of 49 cases with a past history of pneumonia and/or pleurisy, 94 per cent showed adhesions. The cases with a past history of pneumonia and/or pleurisy showed an average area of pleural involvement twice that of the average of comparable age groups without histories of pneumonia and/or pleurisy. Isolated adhesions were more common than diffuse adhesions except in the pneumonia group. The most common location for adhesions was over the lower lobes posteriorly. Microscopically, the great majority of fibrous pleural adhesions were associated with slight fibrous thickening external to the subpleural elastic layer. There was usually no change in the underlying pulmonary tissue. Several other frequently occurring clinical and pathological entities were found to have no apparent relationship to fibrous pleural adhesions.

AMYLOID GOITER. George A. Walker (by invitation), Kansas City, Kans.

Abstract. Fifty-six cases in which amyloid could be demonstrated in the thyroid gland have been reported in the literature. Of these, 35 showed enlargement—true "amyloid goiter." Amyloid deposits occurred as a part of the secondary or generalized type of amyloidosis in 40 cases. The commonest associated primary disease is pulmonary tuberculosis; purulent bronchitis with bronchiectasis or neoplastic processes are less commonly the precursors. Two cases of amyloid thyroid without amyloid in any other organ have been reported (primary amyloidosis of the thyroid).

This paper reported two cases of amyloid in the thyroid gland. The first patient was a white male, 60 years old, who had severe dyspnea due to marked enlargement of the thyroid beginning 3 years before admission to the hospital. At autopsy, tuberculous bronchopneumonia with cavitation and generalized amyloidosis with involvement of the thyroid were found. The thyroid weighed 280 gm., contained very few follicles and showed marked lipomatosis in addition to extensive deposits of amyloid. The second patient was a boy, 16 years old, who developed generalized amyloidosis while under treatment for the active tuberculosis which caused his death. At autopsy, in addition to pulmonary tuberculosis, there was generalized amyloidosis. The thyroid gland weighed 10 gm. and consisted almost entirely of amyloid.

In no reported case has there been any evidence of thyroid deficiency.

Discussion

(Dr. Jack D. Kirshbaum, Chicago, Ill.) Were either of these two cases associated with a severe anemia?

(Dr. Walker) No, sir; none of the cases reported have been associated with anemia, although the clinical and laboratory findings in every case were not available. Another interesting fact is that in spite of extensive replacement of the thyroid parenchyma by amyloid, in no case has there been any evidence of thyroid insufficiency. One of our cases showed an elevated basal metabolic rate, but this was probably due to dyspnea.

PRIMARY SARCOMA OF THYROID GLAND. TWO CASES. Mendel Jacobi and Herman Bolker, Brooklyn, N. Y.

Abstract. Two cases were presented which showed no carcinomatous elements and which clearly arose from the thyroid gland. One of these was of further interest because it also presented the histologic picture of thyroiditis of the struma lymphomatosa type. There is no reported case of this disease with malignant change.

Case 1. A white male, 80 years of age, complained of a mass in the neck enlarging for 5 years, recently causing hoarseness. A stationary mass in the same position in the neck had been noted for 30 years. The basal metabolic rate was plus 22. After operation there was rapid recurrence of the mass. At autopsy there were metastases to the lungs and mediastinum. The mass measured 20 by 15 by 8 cm. It was encapsulated and composed of a multilobular, firm, gray-white tissue. It showed atypical spindle cells arranged in broad bands and whorls. No characteristics of epithelial cells were found. In the pulmonary metastases several areas in suggestive acinar arrangement were shown to be vascular channels.

Case 2. A man, 69 years old, complained of a lump in the neck, difficulty in swallowing and swelling of the neck of 4 months' duration. The mass was in the region of the thyroid gland, was larger on the left and moved with deglutition. The basal metabolic rate was minus 19. The gross specimen was hard, encapsulated, multilobular, gray-white and weighed 352 gm. The microscopic picture varied. Large areas were composed of dense, poorly cellular fibrous tissue, with atrophic acini, and a diffuse lymphocytic infiltrate, often in follicles with prominent germinal centers. Scattered giant cells and areas of squamous metaplasia were present. The characteristics were those of struma lymphomatosa. The neoplastic areas were composed of broad sheets of atypical cells connected by fine processes. Reticulum was prominent and showed condensation in many areas. No characteristics of epithelial cells were found.

Discussion

(Dr. James Ewing, New York, N.Y.) The pictures of this case are quite characteristic of those which have appeared in the literature as sarcoma of the thyroid, of which the majority have been shown, on careful studies, to have definite epithelial structures, and that has been my experience with this characteristic structure—if you look long enough you will find undoubted epithelial areas. However, the authors seem to have been persistent and industrious in excluding such structures. They say they have cut many sections. Then the situation is this: Here is one case in which no epithelial structures are found, which therefore appears to be a sarcoma; but when this one case, or others like it, are observed in which no epithelial structures are found, and which are identical with those in which epithelial structures are found, is it not the conclusion that we have a real case of spindle-celled epithelial tumor of the thyroid?

As for the second case, it seems to me this is a rather characteristic type of small-celled carcinoma of the thyroid, which everyone knows is one of the great puzzles in diagnosis.

DIFFUSE MENINGIOSARCOMA OF THE BRAIN AND SPINAL CORD. S. R. Haythorn and (by invitation) William Shapera and H. C. Stewart, Pittsburgh, Pa.

Abstract. Two cases of fibroblastic meningioma, both of which presented histologic evidences of malignancy, were reported. In the first case the tumor involved the basal meninges and the spinal meninges from the region of the infundibulum to the filum terminale. It ensheathed the entire spinal cord like a stocking and broke into fibrillary branches which surrounded the sheaths of the nerves composing the cauda equina. It apparently arose from the pia and arachnoid, caused distortion and atrophy of the cord, followed the meningeal folds into the fourth ventricle, surrounded the chorioid plexus and formed a small metastasis by extension into a part of the posterior lobe of the pituitary gland.

In the second case the diagnosis was made at autopsy. A hemorrhage the size of a man's fist was found occupying the left supra-orbital space. The hemorrhage was free from the pia but firmly attached to the dura. The region of the second

and third frontal gyri showed pressure distortion from the hemorrhage and an area of softening. On section, a thrombosed arteriole was found in the apex of the wedge-shaped, softened area. This apparently marked the source of the hemorrhage. On microscopic examination, the arachnoid was found to be the seat of a tumor which was exactly the same in structure as that found in the first case. The tumor tissue was attached to the dura but separated from the cortex by the pia. The tumor was diffuse in the sense that it was not encapsulated and spread along the arachnoid zone.

Special stains confirmed the fibroblastic nature of both tumors.

THE RELATION OF POLYPS TO CARCINOMA OF THE COLON. Elson B. Helwig (by invitation), St. Louis, Mo.

Abstract. The colons from 1,358 consecutive autopsies were examined for the presence of mucosal polyps. All other lesions, including both tumors of other types and lesions obviously a result of infection, were excluded from this study. Of the 1,358 colons examined, 128, or 9.4 per cent, contained polyps. Of these 128 cases, 121 were available for detailed microscopic study. After 1 case of familial polyposis was excluded, the remaining 120 cases contained a total of 237 polyps. The 1,358 patients were grouped according to age and the percentage of patients with mucosal polyps of the colon in each age group was determined. The incidence of polyps increased from 3.7 per cent in the first decade to 23 per cent in the eighth decade.

Each polyp was analyzed histologically by step or serial sections for the presence of carcinoma. In determining the presence of carcinoma in a polyp the general criteria suggested by Swinton and Warren were used. These were the presence of two of the following three criteria: (1) anaplasia, (2) irregularity of architecture and (3) invasion. Eleven of the 120 examples of polyps of the large intestine showed foci of carcinoma within a polyp. The incidence of polyps with foci of carcinoma was greatest in the seventh decade. Eight of the 11 cases of polyps with foci of carcinoma were situated in the rectum or sigmoid colon. There were 22 cases of manifest carcinoma of the large intestine among the 1,358 intestines examined. The incidence of manifest carcinoma of the colon was greatest in the seventh decade. Fifteen of the 22 cases of manifest carcinoma occurred in the rectum or sigmoid colon.

Discussion

(Dr. Emmerich von Haam, Columbus, O.) Was the last picture shown one of the cases that died of carcinoma, or was it one of the incidental findings?

(Dr. Helwig) The polyp in the last case was an incidental finding. The first polyp that was shown came from a case in which there was also an obvious or manifest carcinoma.

(Dr. E. T. Bell, Minneapolis, Minn.) I think it might be brought out that while we recognize these as malignant changes in polypi, in talking to the surgeon about it we should not advise a radical operation unless there is invasion of the base, and we should have some kind of name to convey that idea to the surgeon. If one calls this a carcinoma, the surgeon may do a radical operation, which I do not think is necessary when the malignant changes are found only at the surface of the polyp.

(Dr. S. R. Haythorn, Pittsburgh, Pa.) Pathologists are often asked to look at polyps and decide the question of malignancy for the surgeon. Usually such biopsies do not include the base, so that it is impossible to tell whether invasion is present or not. Where the change is early and limited to the superficial portion of the polyp, the decision is a difficult one to make. I was impressed by a paper by Dr.

H. E. Robertson some years ago, in which he reported the examination of 16 polyps removed at autopsy from one individual and found only one of them to be malignant. Unless frank malignancy is found in the section, the surgeon should share the responsibility of a negative diagnosis with the pathologist.

Dr. Bell, do you have anything to say about this?

(Dr. Bell) The same difficulty is encountered by us, and if we do not get the base of the polyp we take no responsibility for the diagnosis. The surgeon must produce the base of the polyp or take the responsibility himself. If the polyp is on a narrow pedicle, it can usually be regarded as benign. Many times we get the tip of the polyp, and we cannot tell whether it is malignant. I think it is dangerous to call polypi malignant merely because we see malignant changes on the surface, since a large percentage of these show no invasion of the base.

(Dr. N. Chandler Foot, New York, N.Y.) I think a very important thing in this connection is for the surgeon, when he sends the biopsy, to send a description of the tumor from which he took it. We seldom get the entire tumor at biopsy. It was only a month or two ago that I saw an instance of this sort, when a small piece of malignant polyp was excised and examined by a local pathologist, and without any knowledge of the gross appearance the diagnosis of carcinoma was made. The surgeon did a combined abdomino-perineal resection of the rectum, and when the specimen came through there was nothing but a so-called adenoma malignum on a long pedicle. The surgeon was very much disturbed when we failed to make the diagnosis of carcinoma of the rectum. The whole thing could have been avoided had the surgeon told the first pathologist where he took his biopsy specimen and the appearance of the tumor. As Dr. Bell says, the condition of the pedicle of these tumors is the most important point of consideration.

(Dr. Helwig) I did not mean to imply or give the impression that these polyps with foci of carcinoma should be regarded as obvious or manifest carcinoma, and the colon resected. I wish to emphasize what Dr. Foot has told you, that if a small portion of the polyp—for instance, the tip—is removed and sent to the pathologist, he cannot do otherwise than make a diagnosis of carcinoma. He cannot understand the structure of the entire polyp from such a biopsy. On the other hand, the pathologist commits a grave error if he does not insist that the entire polyp be removed.

MASTOPATHIA CYSTICA AND MAMMARY CARCINOMA. Carl V. Weller and (by invitation) James W. Logie, Ann Arbor, Mich.

Abstract. The relationship of cystic disease of the breast to mammary carcinoma has long been a subject of controversy, yet practical observation frequently reveals the concomitance of the two conditions. The criteria for the recognition of mastopathia cystica, as applied to the present study, included dilatation of ducts with accompanying fibrosis; areas of "pale-celled" hypertrophy of ductal epithelium, of the type found in the apocrine sweat glands; papillary ingrowths, to varying degree, into cystic ducts, and, frequently, hyperplasia of terminal ducts and acini. Operative material from the breasts of 330 women was reëxamined. Of these specimens, 118 were carcinomatous, and of these 67 showed coexisting mastopathia cystica. Of 212 breasts without carcinoma, 82 showed mastopathia cystica. Construction of a fourfold table and application of the X^2 test to these data indicated that the chance of obtaining such a degree of concomitance, if mastopathia cystica and mammary carcinoma are independent, is less than 1 in 1000. In practical diagnostic experience, carcinoma is frequently found arising in areas of mastopathia cystica. This was true of 13.5 per cent of the cancers of the breast included in this analysis. Upon both statistical and histopathologic grounds a causal relationship between mastopathia cystica and mammary carcinoma must be accepted.

Discussion

(Dr. Francis Carter Wood, New York, N. Y.) It seems to me this is statistically a perfectly sound paper. Certainly, mastopathia cystica is the expression of a reaction to a chronic irritative agent. Also experimental work with animals shows that anything which stimulates proliferation of glandular tissue may induce cancer. Claims have been made that by the injection of theelin, for example, a large number of carcinomas of the breast can be produced in animals. The animal used in many cases was the mouse, certain strains of which are very susceptible to such stimulation. The statement has also been made that a large number of carcinomas may be produced by theelin in the rat, but the growths are the so-called "microscopic carcinomas," and when the animals were kept for a year, no carcinomas developed, except in a very small percentage. Nevertheless, any irritative and reparative process like that seen in the human breast in mastopathia cystica which induces proliferation of epithelium must be considered potentially dangerous.

(Dr. James Ewing, New York, N.Y.) Can Dr. Weller point out any feature of cystic mastitis which is likely to become cancerous? Furthermore, I should like to ask if any conclusion was drawn as to the relation between stagnation of secretion and the origin of carcinoma.

(Dr. Weller) To answer Dr. Ewing as best I can, in the first place, in general we find that the papillary ingrowths into large cysts are seldom the apparent origin for carcinoma. More often the more solid proliferations in the terminal ducts or dilated acini are the apparent sources of the carcinoma, and frequently the only difference between a diagnosis of mastopathia cystica and one of carcinoma depended upon demonstrating infiltrative growth.

I think there was some local relation to stagnation. The retention of material in small cysts may be of some consequence in determining the local development of carcinoma. I intentionally kept away from such considerations in this presentation, for they would require a large group of lantern slides to illustrate the relations.

(Dr. E. T. Bell, Minneapolis, Minn.) I would like to ask if Dr. Weller has ever followed a group of women with cystic mastitis where the breast was not removed, and if so, what were the results? I have followed such a group of around 50 cases for 5 to 10 years and have not seen a carcinoma develop. If we follow Dr. Weller's recommendation, the breast should be amputated for cystic disease. Some of these women are in their twenties. Does Dr. Weller recommend complete mastectomy in young women with cystic disease?

(Dr. Weller) I did not say that women with cystic disease of the breast should have amputation of one or both breasts. I did say such a woman was a candidate for close watching, for frequent clinical examination and check-up. We have not followed a large group such as Dr. Bell inquires about, and I am well aware of the fact that, according to the literature, some follow-ups show a very small proportion of carcinoma after a 5-year period.

Dr. Robert C. Grauer, Pittsburgh, Pa.) Does not Dr. Weller think that the important point in determining whether or not amputation of the breast should be recommended is the age incidence? Because of the importance of ovarian activity in stimulating acinar proliferation, where the younger age group is concerned, there is more chance for malignancy to supervene in these patients than in those of age 50 or more.

(Dr. Weller) That might well be true, but I am still not recommending amputation of the breast for mastopathia cystica, regardless of the age.

(Dr. George H. Hansmann, Milwaukee, Wis.) I do not know much about the formula shown, but I should like to ask if it assumed that all carcinomas when present have as their etiological factor chronic cystic mastitis.

(Dr. Weller) That assumption is not made for a single one. We are considering coincidence or lack of coincidence.

(Dr. Hansmann) The fact is that at that age there might be a coincidence that might be accidental.

(Dr. Weller) That is what we are trying to investigate: whether it might be casual or causal.

THE CYTOLOGIC CHANGES IN CARCINOMA OF THE HUMAN PROSTATE GLAND FOLLOWING ADMINISTRATION OF DIETHYLSTILBESTROL AND DIETHYLSTILBESTROL DIPROPRIONATE. John R. Schenken and Edward L. Burns, New Orleans, La.

Abstract. The effects of diethylstilbestrol diproprionate on carcinoma of the prostate gland were studied in six patients. In five cases biopsies on the same patient were made before and after treatment. In one case studies were made only on tissue removed after treatment. The patients received from 1,100,000 to 1,600,000 international units over periods varying from 23 to 65 days. Control observations were made not only on the 5 neoplastic tissues removed before hormone therapy was started, but also on the tissues of 44 cases of untreated carcinoma of the prostate gland. As compared to the controls, microscopic studies showed, without exception, that treatment was associated with retrogressive tissue changes in the neoplastic cells. In one case identical changes were noted in the metastatic tumor cells of an inguinal lymph node. In all cases the changes consisted of extensive vacuolization of the cytoplasm and pyknosis or karyolysis of the nucleus. In some cases these changes resulted in complete resorption of the cell, leaving clear spaces in the stroma of the prostate gland.

Discussion

(Dr. Emmerich von Haam, Columbus, O.) I should like to ask if the patients showed nausea or any of the other described phenomena following diethylstilbestrol diproprionate, and I should also like to know if the drug was given by intravenous injection, or by what route.

(Dr. Michael B. Shimkin, Baltimore, Md.) I have three questions: Has this form of therapy been used for other malignant neoplasms, particularly those with metastases to bone; second, have the authors any studies to show what changes take place in bony metastases histologically; and third, is there any information available as to what happens in the testes in these cases with particular reference to the interstitial cells?

(Dr. Howard T. Karsner, Cleveland, O.) Has it been possible to check on the output of acid phosphatase?

(Dr. Schenken) In reply to Dr. von Haam's question, there have been some side effects; some have been nauseated and some have vomited, although we have been able to take care of this rather easily by reducing the dosage for a short period of time, after which the dosage is then resumed. The drug was administered intramuscularly.

In reply to Dr. Shimkin, we are trying it on other malignant diseases in which the roentgenologist and the surgeon both have given us a written statement that they have given up. We are not trying it on cases other than those. So far we have nothing definite to report, although there are a few cases in which there may be some encouraging signs.

We have had no bone biopsies as yet, so we do not know anything about the histological changes, but this one patient was studied very thoroughly by the roentgenologist, who reported restoration of the normal bony structure in some areas. No testes have been removed following this treatment, as yet.

Dr. Karsner, we have not determined the acid phosphatase, but such studies are being done now.

THE RATE AND PERIODICITY OF MITOTIC ACTIVITY IN EXPERIMENTAL SQUAMOUS CELL CARCINOMA OF MICE. Charles M. Blumenfeld, Cleveland, O.

Abstract. The growth of tumors is independent; of normal tissues, dependent. In both, growth by increase in the number of cells is accomplished largely by mitosis. Rate and periodicity of mitosis are ultimate expressions of factors regulating it. Therefore a comparison of rate and periodicity of mitosis in a tumor and in the normal tissue from which it arises may shed light on certain fundamental characteristics of the tumor cell. Squamous cell carcinoma of skin was produced by painting the interscapular region of 2-month-old CBA mice with a 0.3 per cent solution of methylcholanthrene in benzene. Malignant tumors and normal skin have been obtained from 36 animals killed at 8 a.m., 12 m., 4 p.m., 8 p.m., 12 p.m. and 4 a.m. Mitotic activity in each specimen was determined by counting the mitoses in 500 fields. All data were subjected to statistical analysis. Mitosis in normal epidermis showed diurnal rhythm, with 247 mitoses per 500 fields at 12 m., and 60, at 12 p.m. The difference is significant. In the tumors, mitotic activity showed no rhythm, remaining, during the 24-hour period, at a level not significantly different from maximum normal activity, *viz.*, 206 mitoses per 500 fields. The following hypothetical explanation is offered: during the process of differentiation and organization to become part of an organ, normal cells acquire a component which, responsive to the stimulus causing function of the organ of which they are a part, shifts them between vegetative and functional activity. Periodic rise in function of an organ causes periodic fall in vegetative activity; therefore, rhythmicity of mitosis. In tumor cells the loss or diminution of this component results in no, or lessened, diversion of cells from vegetative to functional activity. Consequently, the tumor exhibits no periodic depression of its mitotic activity.

THE INCIDENCE OF ALTERATIVE GLOMERULITIS (KIMMELSTIEL) IN ELDERLY PATIENTS. William A. Morningstar (by invitation) and Paul Gross, Pittsburgh, Pa.

Abstract. The patients selected for study were those of 60 years or older who had no clinical or pathologic evidence of glomerulonephritis or significant involvement from pyelonephritis. Of 174 cases investigated, 50 per cent showed no significant glomerular change. There were 30 cases (17 per cent) in which one-half or more of the glomeruli in the slides available for study showed alterative glomerulitis. Cardiac hypertrophy (375 to 540 gm.) was present in 16 of these, while in 11 the cardiac weights ranged between 225 and 350 gm. Although blood chemical studies had not been done on many of these patients, when available the values were within normal limits. From the clinical point of view, none of these patients was uremic.

RENAL LESIONS IN DIABETES MELLITUS. E. T. Bell, Minneapolis, Minn.

Abstract. A microscopic study of the kidneys was made in 460 diabetic patients. Diabetes is about twice as frequent in females as in males. Hypertension (systolic pressure of 150 mm. Hg or higher) was present in 29.3 per cent of those below 50 years of age and in 65.6 per cent of those over 50 years old. Hypertension is definitely more frequent in diabetic than in non-diabetic patients of corresponding age.

Renal arteriolosclerosis was found in 31.8 per cent of those under 50 years of age and in 77.9 per cent of those over 50 years old. The arteriolar lesion is a definite subintimal deposit of a hyaline substance, usually present in a thick layer. Renal arteriolosclerosis is somewhat more frequent in those with large hearts but is present in two-thirds of those with a cardiac weight of 250 to 300 gm. Similarly,

renal arteriolosclerosis increases in frequency as the blood pressure increases, but is found in about one-half of those with systolic pressures below 140 mm. Hg. It may be stated, therefore, that in diabetes, renal arteriolosclerosis may occur independently of hypertension and cardiac hypertrophy.

Intercapillary lesions of different degrees of intensity were found in 20.5 per cent of diabetic patients—15.8 per cent of the males and 26.9 per cent of the females. About one-third of the intercapillary lesions were of spherical shape and the remainder were diffuse. The hyaline masses between the capillaries are formed chiefly from thickening and splitting of the capillary basement membranes. There is always an associated severe hyalinization of the afferent and efferent glomerular arterioles. In chronic glomerulonephritis there is commonly a central hyalinization of the glomerular lobules which, however, may usually be distinguished from the diabetic lesion.

There are no definite clinical features by which diabetes with intercapillary lesions can be distinguished from diabetes without glomerular alterations.

Discussion

(Dr. D. Murray Angevine, Wilmington, Del.) If the lesion is in the wall of the capillary, I should like to ask Dr. Bell why he calls it intercapillary rather than intracapillary.

(Dr. Howard C. Hopps, Chicago, Ill.) I should like to know if there were any changes in the juxtaglomerular apparatus associated with these changes in the arterioles.

(Dr. Grawitz, New York, N.Y.) I should like to ask if there is any correlation between the changes seen histologically and the renal function.

(Dr. Bell) In regard to Dr. Angevine's question, I used the term "intercapillary" because everyone else uses it; the lesion is conspicuously in the center of the lobule and looks as if it were between the capillaries. I do not like that name. I want to emphasize that we may find practically the same lesion in glomerulonephritis and that it consists of a thickening of the capillary walls rather than of a deposit between them. I do not think intercapillary is a satisfactory term.

There are no changes in the juxtaglomerular apparatus.

As to the correlation with renal function, some of the extreme lesions show a depression of renal function. When the changes are very pronounced in all the glomeruli, the patient will have a nitrogen retention. The blood pressure is in general higher with this lesion, and there is also a severe involvement of the afferent arterioles. It never occurs except with a severe involvement of the afferent as well as the efferent arterioles.

STUDIES ON HODGKIN'S DISEASE AND ITS RELATION TO INFECTION BY BRUCELLA.

Wiley D. Forbus and (by invitation) D. W. Goddard, George Margolis, Ivan W. Brown, Jr., and Grace P. Kerby, Durham, N.C.

Abstract. The investigations reported in this paper deal with the problem of a possible etiological relationship between *Brucella* and Hodgkin's disease as suggested by previously published observations from our laboratories. Extended studies on the incidence of antemortem recovery of *Brucella* from 24 typical cases of Hodgkin's disease yielded the following results: *Brucella* was recovered from 9 of 17 cases by blood culture and from 9 of 23 cases by culture of excised lymph nodes; 4 of 23 cases yielded *Brucella* by both blood and node culture and 14 of 24 cases yielded *Brucella* by either blood or node culture. Coincident with the studies on cases of Hodgkin's disease a series of 84 cases of lymphadenopathy, not Hodgkin's disease, was studied by the same cultural methods. This series included a variety of pathological processes. Seven of the 84 cases yielded *Brucella*

either by blood or node culture. Of the 24 cases of Hodgkin's disease, 8 are now unreported, 6 are living and 10 have died of Hodgkin's disease.

The above antemortem studies have been supplemented by studies on 14 fatal cases of Hodgkin's disease (including 6 of the above series), 12 of which were cultured during life and 9 of which were cultured at autopsy. Ten of the 12 cases cultured antemortem yielded *Brucella* by blood or lymph node culture and 6 of the 9 cases cultured at autopsy were positive for *Brucella*. Four of 7 cases yielded this organism both antemortem and at autopsy, and 12 of the 14 cases yielded *Brucella* either before death or at autopsy. None of the cases of Hodgkin's disease showed specific antibody reactions in what we regard as significant titer. In one series of 103 cases, not Hodgkin's disease, studied at autopsy, *Brucella* was recovered once; in a second series of 68 cases this organism was recovered from 10 cases, the combined figure being, therefore, 11 out of 171, or an incidence comparable to that found in the non-Hodgkin's cases studied antemortem.

The strains of *Brucella* obtained at autopsy have been employed in a study of experimental brucellosis in a variety of animals including guinea pigs, rabbits, dogs and hogs. These animals react to intravenous and intraperitoneal inoculations by the production of granulomatous lesions in the peritoneum, and especially in the lymph nodes. The lymph node lesions, particularly in the hog and the guinea pig, are characterized by extensive proliferation of the reticulo-endothelial cells, sometimes so pronounced as to replace almost completely the lymphoid structures. The reaction is sometimes followed by necrosis, and is always accompanied by outpouring of eosinophils and plasma cells. Multinucleated giant cells are typical of the fully developed lesions. In principle and in some of the details the reaction resembles that of Hodgkin's disease. The type of parasitism produced by *Brucella* in the experimental animal is peculiar in that the organisms seem to prefer, if they do not require, an intracellular position for their propagation. This may have important bearing upon the rapidity with which they leave the blood stream when injected intravenously in large numbers and also upon the difficulty of recovering them from animals known to be heavily infected.

Discussion

(Dr. Seaton Sailer, Cincinnati, O.) What effect does radiation have on the bacteria, and on the growth of the lesion?

(Dr. Forbus) We have had no experience with the effects of radiation on this organism.

(Dr. James Ewing, New York, N.Y.) I had the great pleasure and good fortune to have Dr. Forbus bring his material and to study it with him at considerable length, and I will say with a very sympathetic attitude on my part. It is perfectly obvious that he is producing a characteristic granuloma with this organism. After going over much material with him, I made the comment which I still hold: that it just misses duplicating the series of lesions which we see in Hodgkin's disease. I think the lesions on the whole were more acute and exudative. In the early stages a cellular reaction is very marked, but the late fibrosis and the essential characteristics of the Hodgkin's lesion were missing. As I remember it, Dr. Forbus was to gather observations on that and report later as to whether he had any chronic cases which did more thoroughly reproduce the lesions of Hodgkin's disease.

(Dr. Forbus) We have had no opportunity to study brucellosis in experimental animals for a longer period than about 400 days. However, that is a long time in the life of a hog—one of the animals that we employed. Ordinarily this animal lives about 6 months, or rather, it is killed at the end of 6 months, so we do not know what happens in the naturally acquired disease thereafter. Nevertheless, I think you would find it very interesting to look at Dr. Davis's demonstration of spontaneous brucellosis in hogs since one of his cases does show a considerable

degree of fibrosis. In the experimentally produced lesions of the testes, such as appeared in one of our hogs, there was not only fibrosis but also considerable calcification. The organism used to infect this animal was isolated from a scarred and calcified lesion in the spleen of a hog sent to us by Dr. Davis.

(Dr. Howard T. Karsner, Cleveland, O.) Will Dr. Forbus be good enough to recapitulate the results of cultural studies in other regions of the country?

(Dr. Forbus) I know of only one instance of recovery of this organism from a case of Hodgkin's disease in other laboratories than our own. This positive culture was accomplished by Dr. Jeter of Oklahoma City. The organism was grown from a lymph node. Sections of the lymph node and a culture of the organisms were sent to us, and we were able to confirm Dr. Jeter's observations. That patient is now dead, presumably from Hodgkin's disease, but no autopsy was performed. I have no information regarding cultures by anyone else. It should be emphasized that the cases we have studied are not all from our own locality. Of the fatal cases studied at autopsy, two were from distant parts of the country. One was from Cincinnati; another was from Florida—a young boy who made seasonal trips from Florida to Detroit. His home was in the latter place. We have recently studied a case for a period of 6 months—a boy from Washington, D.C.—who finally died of the disease. Unfortunately, we could not obtain permission for autopsy, but we did have lymph node tissues, and there was no question, either clinically or histologically, that the case was one of Hodgkin's disease. After prolonged effort we recovered the organism from the boy's blood. It took 35 cultures to do it.

(Dr. E. T. Bell, Minneapolis, Minn.) Is the milk pasteurized in the part of the country from which the cases came?

(Dr. Forbus) In certain sections, yes, and in others, no.

(Question from audience) I should like to ask if serological studies have been done to prove the presence of antibody.

(Dr. Forbus) Yes, all cases have been studied for the presence of antibodies, and the interesting and puzzling thing is that we have not found them present in any one of the Hodgkin's cases.

(Dr. R. D. Lillie, Bethesda, Md.) Does it not account for the more frequent presence of organisms in Hodgkin's nodes that no antibodies are formed by neoplastic reticulo-endothelial tissue?

(Dr. Forbus) That is a thesis that I would not like to attempt to develop, since we do not look upon the reticulo-endothelial proliferative reaction in Hodgkin's disease as neoplastic in the usual sense of the term. I have no explanation for the absence of antibodies, but I think it is correct to say that this finding is not wholly inconsistent with the presence and pathogenic activity of *Brucella* in the individual.

(Dr. Karsner) With or without Hodgkin's?

(Dr. Forbus) Either with or without.

(Question from audience) I did not hear whether Dr. Forbus said the strain of the organism was porcine or bovine.

(Dr. Forbus) We have recovered *Brucella abortus* only occasionally. The organisms are usually *Brucella melitensis* or *Brucella suis*.

(Dr. George H. Hansmann, Milwaukee, Wis.) Would inoculation of guinea pigs give a positive reaction?

(Dr. Forbus) In one series of studies we inoculated guinea pigs directly with Hodgkin's tissue obtained at autopsy but we never succeeded in producing lesions; but with the organisms that we obtained by cultures of the same tissues we did succeed. These animals were not studied extensively for antibody production but the organisms were consistently recovered from the lesions which were typical of brucellosis.

(Dr. Hansmann) What is the dosage of the organisms injected?

(Dr. Forbus) Approximately 10 billion organisms for the dog and hog. In one case we used 30 billion organisms; with the guinea pig we used about 1 to 3 billion.

(Dr. Emmerich von Haam, Columbus, O.) Did you introduce a stock culture, or do you think the organism has to be isolated from these cases?

(Dr. Forbus) I see no reason why the lesions cannot be reproduced with a stock culture, because the experiments on the dog and hog were done with one of the strains previously used in our guinea pig experiments and that strain had been kept in the laboratory for 6 or 8 months.

(Dr. Israel Davidsohn, Chicago, Ill.) Did you notice the production of antibodies in the experimental animals?

(Dr. Forbus) The typical response occurred in the hog and the dog.

(Dr. Davidsohn) I may add in regard to the production of antibodies in Hodgkin's disease, that in a series of cases studied by me some of the normal antibodies, namely the iso-agglutinins and the agglutinins for sheep red cells, were present in about the same titer as in normal individuals.

ANTEMORTEM AND POSTMORTEM DIFFUSION OF ALCOHOL THROUGH THE BLADDER MUCOSA. Alan R. Moritz and Walter W. Jetter, Boston, Mass.

Abstract. In each of a series of dogs the bladder was isolated from the kidneys by ligation and section of the ureters. Subsequent to this procedure arbitrary initial differences were established between the concentration of alcohol in the blood and its concentration in the urine contained in the isolated bladder. All concentrations were within the normal range in the sense that none of them was higher than might result from drinking an alcoholic beverage. The initial disparity between the concentration of alcohol in the blood and that in the bladder urine was greater in some animals than would be likely to occur in non-experimental conditions. It was found that the bladder urine does not represent an unaltered pool of ureteral urine so far as its alcoholic concentration is concerned. Alcohol passes into the urine by way of the mucosa of the bladder when the initial alcohol concentration of the urine is disproportionately low, and out of the urine by the same route if its alcoholic concentration is disproportionately high. The passage of alcohol through the bladder mucosa may occur in either direction after death and the direction of its diffusion is determined by the disequilibrium in its relative concentration in blood and urine at the time of death. It is not necessary that either the absolute or the relative concentrations of alcohol in urine and blood be different from those which occur normally in order for an interchange of alcohol between bladder urine and blood to occur. In both living and dead animals the passage of alcohol through the bladder's mucosa tends to bring its concentrations in blood and urine closer to, rather than farther from, a state of equilibrium.

The fact that alcohol may pass in either direction through the mucosa of the bladder tends to enhance rather than to detract from the clinical and medicolegal significance of any disproportion which may exist between the alcoholic concentrations of simultaneously collected samples of blood and bladder urine.

CHANGES IN THE MAGNESIUM AND CHLORIDE CONTENT OF THE BLOOD FOLLOWING DROWNING IN FRESH AND SEA WATER. Walter W. Jetter and Alan R. Moritz, Boston, Mass.

Abstract. The pathological changes are frequently inconclusive in establishing the cause of death of persons whose bodies have been recovered from water. In such instances the opinion that death has been caused by drowning is likely to be based in part on the circumstances in which the body was found and in part on the fact that the postmortem examination failed to disclose any other cause of death. It has long been known that the chemical constitution of the blood may be altered

during drowning due to the diffusion of electrolytes through the walls of the pulmonary capillaries. The nature of and the extent to which such changes may occur have not been observed under controlled conditions and the manner in which these changes differ from those which occur after death independently of drowning has not been investigated.

Changes in the concentration and distribution of chlorides and magnesium were studied in four groups of dogs. In the first, the animals were sacrificed by mechanically induced asphyxia (not drowning) and allowed to remain in the open air for as long as 72 hours after death. The rate of postmortem change in these animals was regulated by keeping their bodies at different temperatures. In the second group, the animals were sacrificed by mechanical asphyxia and were then submerged in either fresh or sea water throughout the period of postmortem observation. In the third group, the animals were drowned in fresh water, and in the fourth, they were drowned in sea water. Blood was withdrawn from the right and left sides of the heart at intervals after death and the chloride and magnesium content of both cells and plasma was observed.

In the control animals, it was found that diffusion of both chlorides and magnesium between cells and plasma began soon after death and that within 12 hours the normal antemortem differences in their concentration in cells and plasma had usually almost entirely disappeared. The chloride content of the heart's blood underwent progressive reduction during putrefaction, whereas the magnesium content became progressively higher. Submersion after death did not alter the rate or character of these changes.

In animals drowned in fresh water there was a sharp drop in the chloride content of the blood in both sides of the heart during the agonal period. The reduction was greater than that observed in any of the control animals. The reduction was more pronounced in the left than in the right side. The difference between the concentrations in the two sides of the heart diminished as putrefaction progressed. Marked hemolysis of left ventricular blood was observed within 15 minutes after death.

In animals drowned in sea water it was observed almost immediately after death that both the magnesium and the chlorides in the heart's blood were elevated, the former proportionately more than the latter. The elevation was more pronounced in the left than in the right side of the heart. Hemolysis of blood was delayed and the magnesium content of the stomach was abnormally high.

ALLERGIC REACTIONS TO SURGICAL CATGUT—AN EXPERIMENTAL STUDY. Howard C. Hopps (by invitation), Chicago, Ill.

Abstract. Hypersensitivity to catgut has been produced in rabbits as demonstrated by positive skin tests and by the presence of humoral antibodies. Catgut suture (powdered), sheep intestine and sheep serum are essentially similar in their ability to stimulate the production of antibodies specific for catgut. Heterophil antigen (guinea pig kidney) does not stimulate perceptible hypersensitivity to catgut; neither do individuals with high heterophil antibody titers (following or during the course of infectious mononucleosis) possess demonstrable antibodies for catgut.

Studies of tissue reaction to catgut suture in normal and hypersensitive animals were presented. These included observations on suture implants into the anterior chamber of the eye and histological studies of catgut suture in muscle and subcutaneous tissue. It was concluded that allergy to surgical catgut does not significantly influence early tissue reaction (12 hours to 3 days) to catgut suture.

Discussion

(Dr. Israel Davidsohn, Chicago, Ill.) Did you notice whether there was any evidence of precipitins in patients who had wound separation? It seems to me that the reason why you failed to notice any correlation between the response to catgut and the response to the guinea pig kidney is because the antigen is contained only in the sheep red cells, and I doubt whether it is contained in the serum or in any of the tissues of the sheep.

(Dr. Hopps) Titration of the serum of the patients suffering wound disruption was made only in one case, and in this case there were no antibodies for catgut discoverable. In regard to the heterophil antibody, it was thought that inasmuch as some serum protein was contained in the catgut, there might also be sheep's red corpuscles contained there in sufficient quantity so that a reaction could occur to heterophil antibody.

IMMUNOLOGIC AND TOXIC PROPERTIES OF CASEIN DIGEST AS PREPARED FOR PARENTERAL ADMINISTRATION. Howard C. Hopps and James A. Campbell (by invitation), Chicago, Ill.

Abstract. Is casein digest a safe source of amino acids for parenteral administration? To determine the antigenic or allergenic properties of such digests, rabbits and guinea pigs were sensitized to casein, swine serum and swine pancreas (swine pancreas was used in the preparation of one of the products tested). Animals were also given repeated injections of casein digest; both an acid and an enzymatic hydrolysate were used. Skin tests, precipitin determination and anaphylactic tests failed to demonstrate allergic properties in the products tested.

To determine the toxic properties of casein hydrolysates, reactions of normal smooth muscle strips to casein digests were studied. These studies indicated the presence of histamine-like substances, peptones, or tyramine which might explain the frequently observed reactions of flushing, sensation of warmth and nausea in patients to whom casein digest was administered parenterally. Hydrogen ion concentration and activity were determined as well as their effect on acid-base balance in plasma and in circulating blood. Although these substances were quite acid and markedly lowered the pH of plasma to which they were added, they caused no appreciable change in pH or CO_2-combining power in the circulating blood of human beings. This was considered as evidence of the rapid rate at which amino acids are synthesized into protein.

Evidence was presented from postmortem studies upon individuals who had received casein hydrolysate parenterally that casein digest does not alter the morphology of tissues to a significant degree in those patients who have adequate liver function. The suggestion was made that patients with poor liver function may not be able to synthesize protein at a sufficient rate to conjugate rapidly the administered amino acids and thus may suffer severe acidosis.

OBSERVATIONS ON TUBERCULOUS GUINEA PIGS BEFORE AND AFTER TREATMENT WITH SODIUM P,P'-DIAMINODIPHENYLSULFONE-N,N'-DIDEXTROSE SULFONATE (PROMIN). William H. Feldman and (by invitation) Frank C. Mann and H. Corwin Hinshaw, Rochester, Minn.

Abstract. At the 1941 meeting of the American Association of Pathologists and Bacteriologists there was described an experiment which indicated that a compound known unofficially as promin was capable of exerting a definite effect upon experimental tuberculosis in the guinea pig even though the treatment was delayed for as long as 6 weeks after the infection was introduced. The treated animals lived longer than the untreated, and in the treated animals the anatomic character of

the disease was markedly altered, or recognizable signs of tuberculosis were missing at necropsy. That definite proof might be assembled which would indicate with reasonable certainty that the favorable results seen after treatment with promin had occurred in animals in which lesions of tuberculosis were demonstrably present before treatment was started, this study was made. A total of 51 guinea pigs were inoculated subcutaneously with tubercle bacilli, strain H37RV (dose 0.0005 mg. subcutaneously). Forty-two days later all were found to be sensitized to tuberculin and were divided into two groups. Group 1 consisted of 31 animals; these were not treated. Group 2 consisted of 20 animals; this was the treated group. Beginning with the 42nd day, they received promin (given orally in the food), 300 to 400 mg. per animal each 24 hours. One week after treatment began, biopsy specimens of the liver were obtained from the animals in the treated group and from 12 of the animals in the control group. The experiment was terminated when the last of the controls died. This was 224 days after infection. There were still living at this time 81 per cent of the treated animals. The biopsy material revealed definitely that tuberculosis was present in the livers of both treated and untreated animals at the time treatment was started. These findings were compared eventually with the findings obtained from a study of the same livers at the time of necropsy. Morphologic evidence was offered in proof that lesions of tuberculosis in experimentally infected guinea pigs may regress or actually resolve under the influence of promin.

THE INHIBITORY EFFECT OF OXYGEN ON STARVING PATHOGENIC BACTERIA. Carl E. Cahn-Bronner (by invitation), Chicago, Ill.

Abstract. Some aerobic and facultative anaerobic pathogenic bacteria were cultured under partial starvation by quantitative reduction of the nutrient content of the media. Under such conditions bacteria multiply only when exposed to decreased oxygen tension. Atmospheric oxygen tension inhibits growth. In shake-cultures colonies are formed only in a very thin zone below the surface where the relation between nutrient content and oxygen supply is optimum. The distance of this fine layer of colonies from the surface and the depth of the zone of inhibition below the surface increases inversely with the amount of nutrient and directly with the oxygen tension. This sensitivity to oxygen varies with the temperature, with the pH and with exposure to light. The less the media contain of an easily oxidizable substance, acting as an oxygen acceptor, the more inhibitive is the effect of oxygen. This is due to peroxides formed by the bacteria in abundance of oxygen.

It could not be demonstrated that starving bacteria form more peroxides or less catalase. But it could be shown that minute amounts of hydrogen peroxide are much more stabile in starvation media which are either sterile or contain living bacteria. Some peroxides even may be formed on the surface of such sterile colloidal media by exposure to oxygen. The same phenomenon of layer formation in shake-cultures can be produced by hydrogen peroxide in rich media, as by oxygen under starving conditions. Studies on the bacteriostatic action of hydrogen peroxide revealed that the activity of hydrogen peroxide and the effect of oxygen show the same relationship to variations of both the nutrient content and the pH of the media.

EXPERIMENTAL OBSERVATIONS ON THE BACTERIOSTATIC ACTION OF HYDROXYETHYLAPOCUPREINE UPON DIPLOCOCCUS PNEUMONIAE. Mark M. Bracken and (by invitation) George E. Crum and Albert G. Corrado, Pittsburgh, Pa.

Abstract. Hydroxyethylapocupreine, a quinine derivative made at the Mellon Institute, Pittsburgh, and used in the treatment of pneumonia and malaria, was previously believed to be bactericidal for *Diplococcus pneumoniae*. *In vitro* studies had

demonstrated that no growth occurred in hormone veal broth or in poured blood agar plates inoculated with *D. pneumoniae* type II after 6 hours' incubation in broth containing a 1:2500 dilution of hydroxyethylapocupreine. The present experiments substantiate these findings, but also demonstrate that mice which were inoculated intraperitoneally with the chemical-broth-culture mixture after 6 hours' incubation of the latter, developed fatal infections. Corresponding mice which received the same dosage of chemical-broth-culture mixture after 18 hours' incubation survived. Peritoneal washings of these latter mice were cultured in poured blood agar plates and identical portions were inoculated into fresh mice at intervals of 2 to 24 hours after infection. Many of these blood agar plates showed smooth colonies of *D. pneumoniae,* but all of the corresponding mice survived.

The viability of the *D. pneumoniae* was proven by its growth on the blood agar plates inoculated with peritoneal washings of mice which survived the infection. This demonstrates that hydroxyethylapocupreine, under the conditions of these experiments, is not primarily bactericidal, but is bacteriostatic.

Survival of mice demonstrated to be infected with viable *D. pneumoniae* suggests some reduction of invasiveness or pathogenicity of *D. pneumoniae* after exposure to hydroxyethylapocupreine. It is to be noted that the strain of *D. pneumoniae* used in these experiments uniformly produced fatal infection in mice which were inoculated with one to three microörganisms not treated with hydroxyethylapocupreine in other experiments. Loss of pathogenicity, if such it was, was not maintained. After passage through broth of these "avirulent" *D. pneumoniae,* larger doses produced uniformly fatal infections in primarily healthy mice.

Finally, the experiments demonstrate that bactericidal action shown by *in vitro* methods may in reality be bacteriostasis when studied *in vivo*.

Discussion

(Dr. Howard H. Permar, Pittsburgh, Pa.) I feel that Dr. Bracken has been very wise in employing the word bacteriostatic, and not enlarging on that, because were we to go into virulence, invasiveness and pathogenicity, we might very well get lost. I think he has shown *in vivo* that this chemical has a remarkable effect upon a virulent pneumococcus when transplanted from "primary" to "secondary" mice, at the intervals indicated. Furthermore, it may be that this method could be employed in the study of chemotherapeutic agents which are now being used, and some information of value might be brought forth.

(Dr. M. H. Soule, Ann Arbor, Mich.) Dr. Bracken, do you know whether or not this compound has been used in the treatment of human malaria? The compound mentioned by Dr. Feldman—promin—has been used with rather encouraging results, and inasmuch as we need a substitute for quinine, I am wondering whether the rather optimistic results reported by Dr. Hegner on avian malaria have been confirmed in the human disease.

(Dr. Bracken) This compound has been used in one case of human malaria. The patient was admitted to Mercy Hospital with recurrent chills and fever, and diagnosed as tertian malaria. At the time of the second chill in the hospital, hydroxyethylapocupreine therapy was instituted at a dosage of 120 gr. a day, 15 gr. every 3 hours. The temperature then dropped to normal, and remained normal for the duration of his hospital stay of about 12 days. That was 2 years ago. Since that time there has been no recurrence, so far as I know. This chemical is under observation in the treatment of malaria by the United States Army in the Panama Canal Zone.

THE EFFECT OF ALCOHOLIC INTOXICATION UPON ACQUIRED RESISTANCE TO PNEUMOCOCCAL INFECTION IN RABBITS. Clarence C. Lushbaugh (by invitation), Chicago, Ill.

Abstract. This study was undertaken in an effort to determine (1) if alcoholic intoxication might influence the mechanisms of active immunity, and (2) if large

amounts of antibody would alter the course of infection in the presence of alcoholic intoxication.

In this study 120 rabbits were used. Of these, 56 were actively immunized, 20 passively immunized and 44 were non-immune. Thirty-four of the actively immunized, 15 of the passively immunized, and 22 of the non-immune animals were intoxicated with alcohol throughout the course of the experiment. The infection was started by the intradermal inoculation of 0.1 cc. of a 6- to 8-hour culture of type I pneumococci. Antibody content of the serum was determined by the resuspension-agglutination technic. All the non-immune and the intoxicated passively immunized animals died, while only half of the intoxicated actively immunized animals died. The latter survived longer than either of the former groups. The actively immunized animals had much more antibody than the passively immunized animals. The animals of both these groups survived the infection when the intoxication was withheld. The difference between the lesions of the intoxicated and non-intoxicated animals consisted mainly in the reduced number of leukocytes and the increased number of extracellular bacteria present in the former. The reduction in number of leukocytes and the increase in number of extracellular bacteria was greatest in the intoxicated non-immune, less marked in the intoxicated passively immunized, and least marked in the intoxicated actively immunized animals where the antibody content was greatest.

Conclusions: Alcoholic intoxication reduced resistance to infection by inhibiting the rapid mobilization of the local inflammatory response; consequently the bacteria multiplied too rapidly for the reduced number of leukocytes entering the infected site to destroy them. This effect was opposed by antibody, which, unharmed by alcohol, accelerated the sluggish inflammatory response, enabling significant numbers of leukocytes to reach the area of inflammation, while the antibody effectively checked the spread of the bacteria.

Discussion

(Dr. Alvin G. Foord, Pasadena, Calif.) How much alcohol did these animals get, and what would be the corresponding dose in human cases?

(Dr. Lushbaugh) These animals averaged about 2,000 gm., and it was necessary to give them 50 cc. of 25 per cent alcohol by stomach tube in order to put them in the condition we required, that is, a stupor bordering closely on coma. The alcoholic determination of the blood showed that they had 400 to 600 mg. of alcohol per 100 cc. of blood, which in man would be "dead drunk."

(Dr. Foord) In the first chart you did not have the control of the animals who had alcohol without any pneumococci. Did any of them die from the alcohol?

(Dr. Lushbaugh) I controlled that, but did not put it in. I used 22 animals which received comparable amounts of alcohol, but no pneumococci. One animal did die of a bronchopneumonia, which none of the other animals showed, and 1 died from the effect of the stomach tube being put into its trachea. In other words, the two deaths in this group were accidental and not comparable to the deaths occurring among the immune animals that were infected and similarly intoxicated. Blood cutures showed that the latter had pneumococcic septicemia at the time of death.

THE PATHOLOGY OF SPONTANEOUS COCCIDIOIDAL GRANULOMA IN THE LUNGS OF WILD RODENTS. L. L. Ashburn and (by invitation) C. W. Emmons, Bethesda, Md.

Abstract. Emmons recently reported the finding of fungi in 25 of 105 animals trapped in the desert around San Carlos, Arizona. Of the 25 fungus-infected animals, 7 of the pocket mice *(Perognathus)*, 1 kangaroo rat *(Dipodomys)* and 1 ground squirrel *(Citellus)* showed gross lesions in their lungs at autopsy. The presented report described these lesions and the fungus associated with them.

Microscopically, a total of 20 nodular lesions was found in the 7 mice and 1 kangaroo rat. These lesions occurred most often in the lower lobes and particularly along the anterior border and in that portion of the lung occupying the costophrenic angle. Most of them were superficially located and many caused slight elevation of the overlying pleura. Except for 1 animal, most granulomata measured approximately 1 mm. in diameter. Basically they were formed of fusiform epithelioid cells diffusely and irregularly disposed in the center of the nodules, but often showing concentric arrangement peripherally. A few lesions were formed centrally of adherent, large mononuclear cells. Six granulomata had large central zones of caseous necrosis, varying from 300 to 800 μ in diameter. One such area of necrosis was largely calcified. Fibrosis was present in only 2 lesions, being of moderate degree in 1, and slight in the other. A few lymphocytes were present in the outer wall of most granulomata and 3 had sharply limited, densely cellular lymphocyte mantles varying from 25 to 200 μ in thickness. The multiple small pulmonary lesions present in the ground squirrel were formed of grouped alveoli filled with sheets of large mononuclear cells. In all granulomata there were present few to many fungus cells of varying size. In 2 granulomata (2 mice), 6 fungi showed cytoplasmic cleavage or mature endospores, typical of coccidioides.

Discussion

(Dr. C. L. Davis, Denver, Colo.) It is quite interesting to note that in these rodents the lesions were confined to the thoracic cavity. That is comparable to the 77 cases in cattle studied in the Denver pathological laboratory of the U. S. Bureau of Animal Industry. In no instance have we thus far found coccidioidal invasion outside the thoracic cavity. I am beginning to wonder now, in the light of this information, if the animal species does not have some sort of body resistance to the extent of confining this disease to the thoracic cavity. It is quite different in man, where lesions are likely to be found in any part of the body.

(Dr. Ernest M. Hall, Los Angeles, Calif.) I would like to know if there were any lesions in the tracheobronchial lymph nodes.

(Dr. Alvin G. Foord, Pasadena, Calif.) I think it is of practical interest to us who make examinations of sputum or lung tissue in suspected cases that we should realize if we treat the material with sodium hydrate or acid, as we do tuberculous material, to kill the other organisms so that they will not kill the guinea pig, we will kill the coccidioidal granuloma organism. Smith, who did so much work at Stanford University, found he could kill the streptococci and other organisms in lung tissue and sputum by 0.5 per cent copper sulfate, which allows the coccidioidal spherules to persist, and then by injecting that material and making cultures he was able to obtain the organism. I am sure that in our part of the country we have missed making the diagnosis in the past because we have treated sputum, etc., like tuberculous sputum, killing the coccidioidal organism by so doing.

(Dr. George H. Hansmann, Milwaukee, Wis.) Are you certain that the coccidioides described is the same as that in man?

(Dr. Ashburn) To answer the last question first, the fungus cultures obtained from these animals were not discussed because this phase of the study is incomplete. However, Dr. Emmons obtained *Coccidioides immitis* in culture from 3 of the mice trapped early in the study. These showed no gross lesions and were not saved for histologic examination. These fungi have been inoculated into experimental animals and the pathology is typical of that produced by strains from human cases. In addition to the 3 mice mentioned, fungi were also found in 22 other animals. These fungi were not *C. immitis*. In all of the animals discussed here, there were extra-granulomatous fungus cells, differing slightly from those within the nodules, mainly in their uniform size and staining reaction. The cultures

were obtained by smearing the cut surface of the lung on the culture media. From this it appears that the cultures obtained were from the extra-granulomatous fungus cells.

Mediastinal lymph nodes were seen microscopically in only one animal and this showed no involvement. As to the rest of the body, we examined the pancreas, liver, spleen and kidney in about half of the group, and they were negative grossly and microscopically.

FACILITATION OF INFECTION WITH EQUINE ENCEPHALOMYELITIS VIRUS. Lester S. King, Newtown, Conn.

Abstract. Fifty per cent glycerin injected intraperitoneally, intramuscularly, or intravenously greatly increases the virulence of fixed equine encephalomyelitis virus injected intramuscularly. This increase is approximately 100 times. Evidence was presented that this result is brought about through dehydration, suddenly produced. Gradual dehydration to the same degree, produced by depriving animals of drinking water, is without effect on virus action. The facilitation effect can be produced by injections of very concentrated sodium chloride, but not by distilled water or by 5 per cent glucose. Glycerin has no facilitating effect when the virus is administered intranasally or intra-ocularly

ATTEMPTS TO INFECT VARIOUS STRAINS OF COTTON RATS *(Sigmodon)* WITH THE VIRUS OF POLIOMYELITIS. Marjorie E. Pierce, J. Emerson Kempf (by invitation), and Malcolm H. Soule, Ann Arbor, Mich.

Abstract. A report by Armstrong in 1939 cited the successful transmission of poliomyelitis to the eastern cotton rat *(Sigmodon hispidus hispidus)*. Within 3 months the author had adapted this rat strain to white mice. The importance of having a cheap susceptible laboratory animal for the study of the many unsolved problems of infantile paralysis directed the efforts of many investigators to confirm this work. Our group was most fortunate in having the coöperation of expert mammalogists from the Laboratory of Vertebrate Genetics of the University of Michigan in identifying and obtaining a supply of cotton rats. Armstrong had experienced considerable difficulty in establishing the initial infection in the rodents and it seemed possible that age and species might be important factors. Accordingly, animals were trapped in Florida, Alabama, Louisiana and Arizona. All belonged to the genus *Sigmodon* and were identified as: *hispidus hispidus, hispidus littoralis, hispidus cienegae* and *minimus minimus*. Fertile pairs were mated and breeding colonies established to supply animals of known ages. Those raised in the laboratory have in no manner become domesticated; they are vicious and difficult to handle.

In the experimental work, trapped animals and pedigreed young were treated with suspensions of human cord from seven patients dead of typical bulbar poliomyelitis and monkey cord infected with six human strains well adapted to this host. The infected material was administered intracerebrally, intranasally and subcutaneously to each animal. One group was given one treatment and carefully observed for a period of 90 days. A second group was treated in a similar manner and at the end of 7 days the animals were sacrificed, the brains and cords removed and a new series of rats were treated with suspensions of this material. The procedure was repeated several times in an attempt to adapt the various strains to the rats by serial passage. A third group was treated with virus suspended in the "enteric toxin filtrate" of Toomey. At no time was flaccid paralysis observed in any of the treated animals and the histological examinations made of cord preparations showed no evidence of poliomyelitis.

These negative results suggest that the success of Armstrong was not related to a susceptibility associated with age or species. It would seem more probable that the strain which he established in cotton rats after preliminary adaptation to monkeys is a most unusual strain of the virus of poliomyelitis. In the present state of our knowledge the cotton rat offers no advantage over other rodents as an experimental animal for the study of this disease agent.

Discussion

(Dr. William H. Harris, New Orleans, La.) In connection with his experiments, I would like to ask Dr. Soule if he has had any experience with the murine infection that has been stated to be of spontaneous origin and is quite analogous to poliomyelitis. Furthermore, I would add that we have worked with the murine strain from a primary human source which Dr. Jungeblut has employed in his experiments. It was difficult to transmit when received in ordinary shipment, until finally he kindly sent it air mail and in dry ice. With this latter specimen we were successful in carrying that strain along. The lesions of these animals are quite similar to those of poliomyelitis.

(Dr. Soule) After seeing the histological sections of Dr. Jungeblut at Chicago last spring, we are not convinced he is dealing with a strain of poliomyelitis in his animals. The only strain which we feel confident is entitled to consideration as a cotton rat-adapted strain of poliomyelitis is the Lansing strain of Armstrong.

(Dr. Harris) Are you familiar with the spontaneous disease?

(Dr. Soule) No.

(Dr. Harris) I must state that so far as we can ascertain, the clinical signs and pathological changes of poliomyelitis were present in our animals.

(Dr. Soule) Are you not referring to the strain of poliomyelitis which Dr. Jungeblut claims he has adapted to mice?

(Dr. Harris) Yes; I primarily asked the question concerning the spontaneous disease and next discussed the Jungeblut-adapted strain.

THE PRESERVATION OF BACTERIA, VIRUSES AND PROTOZOA AT −51° TO −53° C. Malcolm H. Soule and (by invitation) Ruth Lofgren and Marjorie E. Pierce, Ann Arbor, Mich.

Abstract. Solid carbon dioxide (dry ice) is quite widely used as a refrigerant for the preservation of disease agents such as viruses, spirochetes and protozoa; forms which cannot be cultured readily on artificial media. In actual practice the dry ice is mixed with ethanol and stored in vacuum flasks along with vials of the germ specimens, or used in a manner similar to ice in specially designed chests. With constant replacement of the refrigerant a temperature of −76° C. may be maintained indefinitely. When adequate attention is given to the freezing and thawing of the infectious material, the microbes not only remain viable but the original virulence is retained to a marked degree. The major objection to this technic is the problem of handling and providing a supply of dry ice. In addition the carbon dioxide may have a toxic action on the germs. Recently a commercial low temperature electric refrigerator (Deep-Freeze) for home use with a capacity of 4 cubic feet came on the market. With the coöperation of the manufacturers one of the machines was constructed with a special mechanical unit and insulation, resulting in the obtaining of an operating temperature of −50° to −53° C. This instrument has been in continuous use for over 12 months and the temperature has not varied more than 2 degrees. Suspensions of infected material containing the following organisms have been placed in the refrigerator: *Spirochaeta novyi, Spirochaeta obermeieri, Spirochaeta kochi* and *Spirochaeta*

duttoni; viruses of eastern equine encephalomyelitis, rabies, poliomyelitis, influenza, vaccinia and *Bacillus coli* bacteriophage; *Trypanosoma lewisi, Trypanosoma brucei, Trypanosoma hippicum* and *Trypanosoma equiperdum.* Before placing the specimens, blood or tissue suspensions in the refrigerator, each was distributed in lusteroid vials previously sterilized by ultraviolet light and quickly frozen in thin layers on the inside of the tubes by immersion in CO_2 and C_2H_5OH at $-52°$ C. At definite intervals following storage, samples were selected and promptly thawed by placing at 37° C. Microscopic examinations were made of the suspensions and animals were inoculated to determine any change in virulence. All of the germs survived and retained their original potency. Certain pronounced morphologic changes were noted in the trypanosomes and to some extent in the spirochetes. The longest storage period evaluated at the time of this report is 148 days. However, the experiment is still in progress and there is no reason to believe the data will be materially altered by subsequent tests. It is apparent that a temperature of $-52°$ C. is just as satisfactory for the preservation of microbic forms as $-76°$ C. The low temperature electric refrigerator has many advantages over the carbon dioxide chest for this purpose.

Discussion

(Dr. S. R. Haythorn, Pittsburgh, Pa.) I should like to ask Dr. Soule if he has tried to preserve *Treponema pallidum* at $-51°$ C.?

(Dr. Soule) Turner was the pioneer in this particular type of work with *Spirochaeta pallida,* and we have confirmed his experiments in every way. We ground up infected rabbit tissue, froze the suspensions quickly and then stored them at $-51°$ C. This material produced typical infections in rabbit testes. We have found a temperature of $-50°$ C. just as satisfactory as $-76°$ C., the temperature used by Turner, for the preservation of syphilitic material.

(Dr. William H. Feldman, Rochester, Minn.) Have you applied this method to any of the strains of mycobacteria?

(Dr. Soule) The mycobacteria are so easy to culture and maintain that we have not applied this method to them. The problem we have is with trypanosomes, spirochetes and viruses—forms hard to cultivate. If we get a group of viruses, we like to have them kept in a manner which will maintain their virulence just about at the level at which we received them.

(Dr. William H. Harris, New Orleans, La.) Dr. Soule spoke about the altered morphology of the trypanosomes after freezing, and yet referred to their subsequent virulence in animals. The original morphological characteristics most likely reappeared on re-injection in animals, did they not?

(Dr. Soule) Yes.

(Dr. Harris) Then their nature is not really changed by this process?

(Dr. Soule) That is a problem that has bothered us, particularly with the protozoa. There is no reason to believe that we change their nature by the freezing process. With malarial blood the changes are not great.

(Dr. Harris) Then it is not a really serious problem? The process of freezing, after all, really answers the primary purposes of preservation, inasmuch as there occurs restoration of all the biological characteristics through subsequent animal inoculation.

(Dr. Soule) It would be more encouraging if we could control the morphological changes in the freezing and thawing processes. It is extremely important to freeze very rapidly.

THE HISTOLOGY AND TOPOGRAPHY OF THE BRAIN LESIONS OF EQUINE ENCEPHALOMYELITIS IN MAN. James H. Peers, Washington, D. C.

Abstract. In the summer of 1941 a severe epidemic of encephalitis occurred in North Dakota and neighboring states. The following description of the pathology of this disease was based upon examination of 13 brain specimens, in 4 of which the virus of the western type of equine encephalomyelitis was demonstrated by animal inoculation. This virus may produce in man a widespread and severe encephalitis, characterized by nodular microglioses, spongy areas of partial disintegration of the ground substance and perivascular round cell infiltration. These lesions constantly appear in greatest number and severity in the anterior part of the putamen and caudate nuclei, and markedly diminish posteriorly. In the brain stem, lesions are evenly distributed in the tegmentum and base, apparently independent of anatomic and functional units. A variable number of lesions are also present in the anterior cerebral cortex, and in the cortex and the roof nuclei of the cerebellum. The disease process is predominantly located in the gray matter, but scattered foci of microgliosis with partial demyelinization are seen in the white matter. The meninges contain only a scanty round cell infiltration. No inclusion bodies were observed.

Discussion

(Dr. Lester S. King, Newtown, Conn.) I think it rather remarkable that the histological sequence which Dr. Peers describes differs so extremely from what is found in the eastern type of equine encephalomyelitis. I have had considerable opportunity to study lesions in horses, and experimentally in guinea pigs and mice, and in human material. In the eastern type of the disease, the characteristic lesion is a focus of polymorphonuclear leukocytes, sometimes around a vessel, and sometimes diffusely through the tissue, independent of a vessel. In addition, the blood vessels often show marked inflammatory changes in their walls. The glial nodules are prominent in this type also. The spongy areas which appear in the western type are not so prominent in the eastern type, and where they do occur, they appear to be independent of any inflammatory change. From the differences in pathology we cannot draw any conclusion as to the similarities or relationship of the two viruses. That can only be determined by serological and immunological work, but I wish to emphasize again that the types of pathology are quite different in the disease which Dr. Peers has discussed and in the eastern disease with which I am familiar.

(Dr. Peers) I want to thank Dr. King for bringing out these points. I, myself, have had no opportunity to see any of the eastern material. I have had some promised me, and I shall be glad to look for these differential features which Dr. King mentioned.

COMPARATIVE STUDIES OF MENINGOPNEUMONITIS VIRUS, PSITTACOSIS OF PIGEON ORIGIN AND PSITTACOSIS OF PARROT ORIGIN. H. Pinkerton and (by invitation) V. Moragues, St. Louis, Mo.

Abstract. Comparative morphological, histological and biological studies suggest a close relationship between the meningopneumonitis virus of Francis and Magill and a virus recovered by Pinkerton and Swank from thiamin-deficient pigeons. Both of these viruses are morphologically identical with typical psittacosis, and it seems probable that they are biologically modified strains of psittacosis. They both differ from typical psittacosis in that they show much greater pathogenicity for the pigeon after intracranial injection, and fail to produce hepatic necrosis after intraperitoneal injection in mice. A virus recently isolated from human cases of atypical pneumonia by Eaton, Beck and Pearson behaves similarly in mice, and may also be closely related to these two viruses. Six other strains of

psittacosis of pigeon origin showed a similar increased pathogenicity for pigeons by the intracerebral route, as compared with ten strains of psittacosis viruses of parrot origin. The viruses of parrot origin, however, commonly produced latent infection in pigeons even when clinical illness was not evidenced.

For the isolation of psittacosis of pigeon origin from human sputum, the intracranial injection of mice or pigeons may be essential, although it is probable that the intranasal injection of mice would be successful. The intraperitoneal injection of mice may give negative results.

The virus of lymphogranuloma venereum is apparently non-pathogenic for pigeons by the intracerebral route.

POSTMORTEM EVIDENCE OF ACUTE INFECTION IN UNEXPECTED DEATH IN INFANCY. Jacob Werne, New York, N. Y.

Abstract. This presentation emphasized the occurrence of pulmonary lesions in a group of infants dying unexpectedly, in whom the gross autopsy findings were inadequate for the reasonable certification of death. In 50 such cases collected over a 10-year period from a routine medicolegal autopsy service in the Borough of Queens (in the Office of the Chief Medical Examiner of New York City), bacteriological study was possible in 28. The histological findings were sufficiently uniform in all to warrant the inclusion of the remaining 22 cases.

The organisms encountered, and the number of times, were as follows:

Streptococcus viridans	12
Streptococcus, non-hemolytic	9
Bacillus coli	9
Staphylococcus aureus, non-hemolyzing	8
Streptococcus hemolyticus	7
Staphylococcus aureus, hemolytic	3
Streptococcus liquefaciens	2
Streptococcus faecalis	2
Streptococcus foetidus	1
Salmonella derby	1
Brucella melitensis	1
Friedländer's bacillus, A	1
Pneumococcus, type 14	1
Pneumococcus, type 21	1
Pneumococcus, not type-specific	1

The majority of sections of lung showed the occurrence of bronchitis, peribronchitis and peribronchial pneumonitis; mononuclear cells predominating. Extreme capillary congestion, pericapillary and intra-alveolar edema, and edema and cellular infiltration of subpleural and larger septal tissues were frequently found. In a smaller number of cases the presence of a diffuse interstitial reaction without evidence of primary bronchial inflammation suggested other than a respiratory portal of entry. Such was the picture in the cases showing dermatitis and in one where the presence of a microscopic pylephlebitis suggested an intestinal portal of entry.

Taken in conjunction with postmortem bacteriological findings, the presence of the lesions described, illustrated by lantern slides, supports the conclusion that acute infection was the cause of death in these cases.

Discussion

(Dr. Howard C. Hopps, Chicago, Ill.) In one of the sections shown there appeared to be macrophages filled with large fat droplets. Was lipoid pneumonia present in these cases?

(Dr. Werne) The section in question shows very marked pericapillary edema, and I believe the droplets are hydropic rather than fat. In many of these cases routine fat stains were done of lung and liver. In no instances was evidence of lipoid pneumonia or reaction to foreign body found.

(Dr. E. T. Bell, Minneapolis, Minn.) I am glad to hear Dr. Werne's paper. I think if more studies like this were made we would seldom be called upon to make the diagnosis of status lymphaticus.

(Dr. Werne) I agree with Dr. Bell as to the inadvisability of making a diagnosis of status lymphaticus. I recognize hyperplasia of lymphatic tissues and the presence of an uninvoluted thymus solely as anatomic findings, but not in themselves as adequate explanation for death.

(Dr. Wiley D. Forbus, Durham, N. C.) I think this a very important subject from the medicolegal point of view. Almost all of us have had the experience of being called to explain such deaths in children as have been described in this paper. If one is not familiar with the rapidity with which death can result from overwhelming bacterial infection, one may find himself in great difficulty and embarrassment in dealing with cases of "sudden death" and be led quite far astray by incidental autopsy findings that would be wholly ignored under different circumstances. This presentation is a timely one.

(Dr. O. O. Christianson, Peoria, Ill.) This paper interested me very much because lately I had a similar case. A child of 3 months had received an overdose of atropine because the druggist had made an error in filling the prescription. Two days later the child became very ill and died. Findings at autopsy were quite negative except for what was considered to be terminal aspiration pneumonia. However, I was surprised to find that cultures of the lungs, spinal fluid and blood revealed a heavy growth of *Streptococcus viridans*. The histologic changes in the lungs were identical with those described by Dr. Werne. In this case the parents' lawyer is attempting to prove that the child died from an overdose of atropine. However, I am certain that the autopsy findings will frustrate this attempt.

(Dr. George H. Hansmann, Milwaukee, Wis.) I wonder why this is limited to children; it happens in adults, too, with overwhelming infection. One is often discouraged in routine autopsies to find the blood cultures are not very valuable, but as here demonstrated, they are of occasional importance. On the other hand, I wonder what the mechanism is? Do some of these children die of asphyxia due to the aspiration of milk? I wonder if anoxemia sufficient to cause asphyxiation occurs from the aspiration of milk or food which had been regurgitated. I noted that bile was found in the terminal bronchioles.

(Dr. Werne) Cases of fulminating infection are also met with in adults, but far less frequently than in children, and very rarely without some gross evidence upon the basis of which death may be reasonably certified. For that reason their occurrence constitutes a relatively minor medicolegal problem and was not considered in this presentation. With regard to the mechanism of death, there are some in whom aspiration of stomach contents is either a terminal or a significant terminating phenomenon. In the majority of cases the pulmonary edema must be regarded as the strongest evidence of a mechanical basis for death. The quantitative measurement of the influence of shock, for which the bacterial findings with their associated lesions are evidence, is a difficult one with postmortem material.

(Dr. William H. Harris, New Orleans, La.) I did not hear any description nor did I see any illustration of other organs in these cases. Was the entire pathology present limited to the lungs? Prior to unexpected death, were these children regarded as entirely normal? I ask this because while a death may be unexpected, the subject may be, of course, primarily abnormal. From what I could hear, it

appeared that these children were normal. The bright red color of the lungs shown and the emphasis upon lymphoid cell distribution and edema in the interstices appear to me to be matters of observation rather than definite explanation.

(Dr. Werne) The time has limited my presentation to a discussion of the pulmonary lesions, which I consider the most apparent. Many of my cases show glomerular lesions, foci of cellular infiltrate in heart and liver, and extreme hyperemia and hyperplasia of lymphatic tissues. These will be reported in a subsequent presentation.

(Dr. Seaton Sailer, Cincinnati, O.) Is this prominent interstitial edema which has been described regarded as a serous inflammatory exudate, or is it a cold process—a simple edema following the shock of overwhelming toxic absorption?

(Dr. Werne) I believe that several factors are involved. We have direct evidence in some of these cases of bacterial injury in the form of capillaries distended with bacterial colonies. In others we find, as illustrated, bacterial colonies in the bronchioles. In association with both types of occurrence there is a significant reaction. Extreme capillary congestion, pericapillary and intra-alveolar edema and marked edema of the subpleural and larger septal tissues are noted. The association with this edema of significant cellular infiltration, mononuclear in the main, speaks for an inflammatory origin, I believe. Part of the picture is certainly referable to the edema that we now recognize as a histopathological expression of shock. The shock in this instance, I believe, is referable to the fulminating bacterial process.

(Question from audience) How many hours after death were the autopsies done, and were any cases used as controls?

(Dr. Werne) In most instances the bodies were refrigerated for 4 to 6 hours after death, and the autopsies performed either the same day or the following morning. We have been controlling our bacteriological findings in this group by routinely culturing the viscera of infants and children dying of violence, and in the last four cases, which happened to be drownings, the lungs were sterile in all but one instance, in which *Staphylococcus albus* was recovered. There were no visceral lesions in these control cases.

MEDIASTINAL SYMPATHOGONIOMA. Seaton Sailer, Cincinnati, O.

Abstract. A colored female, 65 years old, was admitted to the accident room of Cincinnati General Hospital in coma. Three days before admission she had complained of severe headache, dizziness and weakness. Upon admission, spinal fluid cultures and smear were positive for *Haemophilus influenzae*. Death occurred 2 days later and autopsy showed an extensive purulent leptomeningitis involving the entire brain and spinal cord. Some bronchopneumonia was present in the left lower lobe. Occupying the anterior superior mediastinum and overlying and bound to the superior vena cava by fibrous adhesions, was a well encapsulated, solid, firm tumor 5 cm. in diameter. Portions of this were also attached to the mediastinal surface of the right lung and the eparterial bronchus by fibrous bands. On section the tumor capsule was thick and partially calcified. The parenchyma was reddish brown and rather soft. Microscopic sections showed the histologic structure of a sympathogonioma with closely packed, deep-staining, small, round and oval cells showing some attempt at rosette formation. There was no evidence of metastasis. The few cases of this condition found in the literature were briefly reviewed.

NEUROBLASTOMA OF THE MEDIASTINUM CONTAINING PHEOCHROMOBLASTOMATOUS ELEMENTS. H. R. Wahl and (by invitation) David Robinson, Kansas City, Kans.

Abstract. A brief review of sympathetic nerve tumors arising in the thorax was given with report of a case occurring in a child of 4 years. Forty-one related

tumors were found in the literature, including 27 ganglioneuromata, 13 neuroblastomata and 2 paragangliomata. This tumor involved the upper right mediastinum with direct extension into the upper right lobe and metastasis to the cervical lymph nodes and to bones, especially the skull. The main tumor mass was composed of both undifferentiated and differentiated elements. The former were neuroblasts or sympathicoblasts and formed metastases to the bones. The latter were larger, had more cytoplasm and formed a tissue that was partly encapsulated and simulated that frequently described in the medulla of the adrenal gland as a paraganglioma or pheochromocytoma. This portion of the tumor showed many giant cells, often multinucleated. An argentaffin reaction was obtained but the chromaffin reaction was inconclusive, probably because of unsatisfactory fixation. A few immature ganglion cells were present.

Of the three types of sympathetic nerve tumors reported, various mixtures have often been noted. The pheochromocytoma tends to be found in the pure state more often than either the ganglioneuroma, the neuroblastoma, or its more differentiated form, the sympathicoblastoma. This case is unique in that a large part is definitely undifferentiated and malignant and made up of neuroblasts and sympathicoblasts, while the differentiated portion, instead of showing the usual ganglion cells and nerve fibrillae, is made up of cuboidal, polyhedral and giant cells such as are seen in the true pheochromocytoma.

PHEOCHROMOCYTOMAS: MORPHOLOGY AND FUNCTIONAL ASPECTS (A STUDY OF FOUR CASES). Eleanor M. Humphreys, Chicago, Ill.

Abstract. The pheochromocytomas studied were found in the adrenal glands of four women whose ages were 51, 47, 41 and 36 years. They demonstrated well the pathologic physiology and morphology of this interesting endocrine tumor. In one case, a fall initiated a fatal attack. Although this woman's main symptoms were like those of shock, her systolic blood pressure fluctuated between 130 and 268 mm. of Hg for 48 hours, and terminally her temperature reached 109° F. The only noteworthy findings at autopsy were the small tumor and an oblique crack in the neck of one femur. Two of the other patients had been near death from pulmonary edema. All three had attacks associated with paroxysmal hypertension, abolished by excision of their tumors. Most of the manifestations of attacks were strikingly like those effected in man and animals by suitable doses of adrenalin. One exception was bradycardia, which often replaced or alternated with the expected tachycardia. It was interesting that two of the patients had hypersensitive carotid sinus reflexes of the vagal type.

One case illustrated a common diagnostic problem. Thyrotoxicosis was suspected because of the patient's nervousness and vasomotor instability, her exophthalmos, and basal metabolic rates from plus 30 to plus 70. All four tumors were encapsulated and quite vascular. One was cystic; another focally necrotic and hemorrhagic. They weighed from 13.5 to 75.0 gm. Slices immersed in 3 per cent potassium dichromate solution quickly changed from gray-pink to brownish black. Assays (chemical and biological) indicated the presence of from 0.6 to 3.0 mg. per gm. of adrenalin-like substances in various samples.

Microscopically the cell types varied so greatly, even within one and the same tumor, that morphologic criteria for identification had limited value. The best criteria were the gross and microscopic chromaffinity, the assays, and, in three of the cases, the abolition of attacks once the tumor was excised.

Discussion of Papers by Drs. Sailer, Wahl and Robinson, and Humphreys

(Dr. Jack D. Kirshbaum, Chicago, Ill.) Was an adrenalin-like substance demonstrated in the blood before operation in any of these cases?

(Dr. Humphreys) It was not. The only case in which that has been done was that of Beer, King and Prinzmetal (Beer, Edwin; King, F. H., and Prinzmetal, M. *Ann. Surg.*, 1937, 106, 85–91), unless others have been reported quite recently.

(Dr. Howard T. Karsner, Cleveland, O.) It seems to me that the preceding three papers belong in a group and it would be unfortunate if they were not discussed. The mediastinal sympathogonioma reminds me of the case of Frost and Wolpaw (Frost, T. T., and Wolpaw, S. E. *Am. J. Cancer*, 1936, 26, 483–492) in which a tumor in the superior pulmonary sulcus with Horner's syndrome proved to be a sympathogonioma rather than the customary carcinoma.

Dr. Wahl modestly made no reference to the fact that in 1914 he published a paper on neuroblastoma which to my mind was a masterful presentation of the subject at that time. The tumor he reported today illustrates the fact that tumors of the sympathetic system may show departure from cell type. Indeed, multiple sympathetic tumors may be of different orders of differentiation in different situations.

Pheochromocytomas may also show variations in cell picture. I should like to have Dr. Humphreys express her views about the following idea. In these tumors of the adrenal there are usually cells which, because of arrangement and of lipid content, appear to represent adrenal cortex. I have not read of, or seen, these cortex-like cells in pheochromocytomas of the thorax or those situated in the region of Zuckerkandl's organ. It is unfortunate that so many patients with tumors of endocrine organs are operated upon without having an assay of hormones. Of course the manifestations of pheochromocytomas are like those due to a large dose of adrenalin. My recollection is that although Prinzmetal demonstrated a pressor substance in the blood, he did not prove it to be epinephrine. The marked drop in blood pressure that is likely to occur as these tumors are removed at operation points strongly to the production of a pressor substance by the tumors. In one of our cases, the patient received continuous infusions of adrenalin for nearly 18 hours before he maintained blood pressure above shock levels. Evidently it took this time before the opposite adrenal (presumably atrophic) functioned adequately. The diagnosis of these tumors and the effects of their removal are a tribute to the achievements of modern medicine.

(Dr. George A. Walker, Kansas City, Kans.) Dr. Karsner referred to the fact that these pheochromocytomas sometimes contain cells suggesting an origin from the adrenal cortex. We saw a case recently in Kansas City, a child of 3 years, who had as the outstanding clinical manifestation of the disease a very well marked sexual precocity, with the adult type of distribution of axillary and pubic hair, and adiposities of the female type. Associated with these was the usual picture of paroxysmal hypertension. Air injection was carried out and revealed a tumor in the region of the adrenal gland. This was removed surgically. This patient also showed a very marked drop in blood pressure during the operation and was carried through by the administration of chloride and adrenalin. In the months that have passed since the operative removal of this tumor, the hypertension has receded; the patient has shown no instability of the blood pressure and there has been a gradual recession of the precocious sexual development. Therefore, this patient had a tumor whose histology was that of a pheochromocytoma but in addition to the usual paroxysmal hypertension associated with such tumors, this patient had also well marked precocious sexual development.

(Dr. Humphreys) One of the things I would like to speak about is the occurrence of the cortical types of cell. I am quite sure there are cells in at least two of the tumors which I illustrated which look very much like cortical cells. I believe Dr. Weller pointed out some time ago (quoted in Coller, F. A.; Field, Henry, Jr., and Durant, T. M. *Arch. Surg.*, 1934, 28, 1136–1148) that in a tumor he had studied some of the cells very closely resembled those of the cortex.

However, it is not always easy to differentiate cortical cells, lipoid-filled macrophages and medullary cells. In one of these tumors one section had definitely chromated cells which in non-chromated sections looked like cortical cells.

As for the statement by Beer, King and Prinzmetal about epinephrine, they were naturally extremely cautious. They used the denervated rabbit's ear perfusion method and the reaction was blocked by ergotamine. On the other hand, further evidence was provided by the actual recovery of pure epinephrine from a tumor by Kendall or someone in his group at the Mayo Clinic (Kelly, H. M.; Piper, M. C.; Wilder, R. M., and Walters, Waltman. *Proc. Staff Meet., Mayo Clin.*, 1936, 11, 65–70.)

It is unfortunate that in the blood of the patients of this report the demonstration of adrenalin was not accomplished. An attempt was made by Dr. Louis Leiter in one case, without success. However, the patient on whom this attempt was made had only occasional attacks. I think if the blood of the patient whose chart I presented, showing the persistent mild hypertension and multiple daily attacks, had been investigated, we might have been more successful.

CORTICAL CARCINOMA OF THE ADRENAL WITH ADRENOGENITAL SYNDROME ASSOCIATED WITH AN ADENOMA OF THE PITUITARY. Jacob M. Ravid, Brooklyn, N. Y.

Abstract. A girl, aged 13½ years, was admitted to the Israel Zion Hospital with an 8 months' history of amenorrhea, hirsutism, pain in the abdomen and change in voice. Examination revealed obesity, coarse hair over the face and body, hypertrophied clitoris, a mass in the right lower quadrant and hypertension. On operation a mass was removed from the site of the right adrenal. It weighed 195 gm., was encapsulated, nodular and retained the shape of the adrenal. Microscopically it showed a typical cortical carcinoma of the adrenal. Postoperative radiotherapy was given. Within 9 months there was an almost complete *restitutio ad integrum.* A report of this case at this stage of recovery was made by Dr. Joseph Tenenbaum (*J. Urol.*, 1939, 42, 277–287). In the 14th month, however, there began a recurrence and recrudescence of all previous symptoms. In addition, there were hyperglycemia, glycosuria, hypercholesterolemia and marked hypertension. The urine was negative for estrin and prolan. Pyodermia developed. In the 18th month after operation the patient died.

Autopsy showed a recurrent tumor at the operative site and widespread metastases in the right kidney, liver, pancreas and lungs. The histological picture was, in the main, similar to the original tumor. Ponceau-fuchsin stain of the tumor by the Broster-Vines method (Broster, L. R., Vines, H. W. C., *et al.* The Adrenal Cortex Intersexuality. Chapman & Hall, Ltd., London, 1938) was negative. A most interesting finding was a miliary papillary adenoma of the anterior lobe of the pituitary, which is probably related to the basophil variety, and some changes in the basophils.

The case illustrates the following features: (1) From the gross and microscopical appearances, the impression was gained that the carcinoma probably developed on the basis of a preëxisting adenoma. (2) Unlike most other "functioning" tumors, it is the adrenal *carcinoma,* rather than the adenoma, which most frequently exhibits functional activity. (3) The association here with an adenoma of the pituitary, coupled with some changes in the basophils, is a finding which requires further elucidation in the present state of confusion between the corticoadrenal syndrome and pituitary basophilism.

ADRENAL CORTICAL NEOPLASMS: ADENOMA AND CARCINOMA ASSOCIATED WITH THE CUSHING SYNDROME. Eleanor M. Humphreys, Chicago, Ill.

Abstract. This study permitted comparisons between early and late phases of the Cushing syndrome. The patients, two women aged 47 and 29 years, had tumors

of the adrenal cortex. The first had symptoms for 4 months and died after resection of a carcinoma which had metastasized to the lungs and liver. A small adenoma was found at the autopsy of the second patient, who had been ill for 3½ years. Neither one had other endocrine tumors or hyperplasias. However, the hypophysis of each contained hyalinized basophils of the type described by Crooke, and rarely observed save in patients with the Cushing syndrome. Both women had characteristic obesity, facial hirsutism, prominent eyes, purple abdominal striations, husky voices, hypertension and amenorrhea without masculinization of the genitalia. Obesity was less typical in the previously overweight older woman, and became less striking before the other woman died. Chemical examination of the blood of the patient with the briefer illness demonstrated impaired glyconeogenesis; increased sodium and decreased potassium and chlorides, and alkalotic values for CO_2 and pH. As pointed out by others (McQuarrie, Irvine; Johnson, R. M., and Ziegler, M. R., *Endocrinology*, 1937, **21**, 762–772, and Willson, D. M.; Power, M. H., and Kepler, E. J., *J. Clin. Investigation*, 1940, **19**, 701–707), this electrolyte pattern is, save for the chlorides, the reverse of that found in Addison's disease. The younger patient had had symptoms for 2 years when first seen, and her subsequent course was that of progressive renal failure terminating with uremic acidosis. Autopsy findings were generalized arteriosclerosis; contracted kidneys; marked skeletal rarefaction with deformities from compression and fractures, and atrophy of endocrine organs, including the non-neoplastic parts of the adrenal cortices. This cortical atrophy and the hyalinization of hypophyseal basophils were the endocrine lesions shared by both patients. Both the carcinoma and its metastases and the adenoma had regions closely resembling the normal adult adrenal cortex. Mitoses were numerous in the carcinoma and brown pigment like that of the reticular zone was abundant in the adenoma.

Discussion of Papers by Drs. Ravid and Humphreys

(Dr. Robert C. Grauer, Pittsburgh, Pa.) I would like to ask Dr. Ravid and Dr. Humphreys whether urinary assays were done to determine the relative proportion of dehydro-iso-androsterone and androsterone. This may help to differentiate the two types of adrenal cortical tumor; the one that expresses itself entirely as an adrenogenital syndrome, from the adrenal cortical tumor which produces the manifestations of the Cushing syndrome. We know that there is a normal relative proportion of dehydro-iso-androsterone to androsterone of about 5 per cent to 95 per cent. If an adrenogenital syndrome supervenes as a result of a cortical tumor, that proportion is distributed so that there is about 40 per cent dehydro-iso-androsterone and 60 per cent androsterone excreted. Where we are confronted with a confusing tumor, one in respect to which the clinical manifestations are at variance with the pathological findings, it might be of great value to determine the relative proportions of dehydro-iso-androsterone and androsterone.

(Dr. Ravid) No assays for the androgenic hormones were done, but only for estrin and prolan, and these were negative.

(Dr. Humphreys) The first case, that of the woman with the benign tumor, was studied some years ago (1935) and the only assays for hormones were made on the urine well after the onset of the syndrome, when the patient was developing renal insufficiency. There was no variation from normal in regard to the estrogenic or male sex hormones (10 gamma per day of theelin and 14 international units of male hormone). The other case is a very recent one, and I know that a part of the tumor was to be assayed.

(Dr. Grauer) I meant urinary assay; not assay of the tumor itself.

(Dr. Humphreys) I believe urinary assays are being done.

GYNANDROBLASTOMA OF THE OVARY. William C. Black, Denver, Colo.

Abstract. An ovarian tumor was removed surgically from a white woman, 24 years old, who exhibited hirsutism with male voice and beard and hypertrophy of the clitoris, these changes having developed during the previous 5 years. Menstruation, although varying in the amount of flow, had always been regular with 28-day intervals until the last 4 months before hospitalization. Since then she had flowed for 5 to 7 days about every 2 weeks. Normal menstruation followed operation. She is well at present, 2½ years later, but there have been only slight changes in the hirsutism and voice, and no change in the clitoris. Histologic examination of the ovarian tumor disclosed a heterogeneous structure with extensive necrosis. In part the form and arrangement of tumor cells corresponded to that of the sarcomatoid form of granulosa cell tumor, but other portions consisted of imperfect tubules with isolated cells and groups of cells containing much lipoid material and resembling the interstitial cells of the testis. The tumor lay in the medulla of the ovary. Beneath the tunica albuginea, which formed the tumor capsule, there were marginally located large tubular glands with tall columnar epithelium including numerous mucus-secreting goblet cells.

I believe that this tumor should be classified as a gynandroblastoma, a term suggested by Robert Meyer (*Beitr. z. Path. Anat. u. z. allg. Path.*, 1930, **84**, 485–520). A similar case, with a review of the literature, has been reported by W. P. Plate (*J. Obst. & Gynaec. Brit. Emp.*, 1938, **45**, 254–257).

Discussion

(Dr. Walter Schiller, Chicago, Ill.) The valuable presentation of Dr. Black deserves special interest, since among the rare ovarian tumors the gynandroblastoma is one of the rarest. The term gynandroblastoma was coined by Robert Meyer in 1930, to signify an ovarian tumor composed of granulosa cell tumor tissue and of arrhenoblastoma tissue developing from a mesenchyme of indifferent cells which, when developing in a tumor, differentiate partly in the male and partly in the female line. Thus a tumor originates which in the field of neoplasms corresponds to the hermaphroditic gonad. Similarly as the hermaphroditic gonad is composed of male and female normal parenchyma, the gynandroblastoma is composed of male and female tumor tissue. Robert Meyer only suggested and anticipated this entity, but found no sufficient evidence to classify his own cases in the new group. Since the appearance of his paper, only a few cases have been published. Plate, in 1938, published 1 case and reviewed 12 cases from the literature, but of these cases only a small minority comply with the definition of Robert Meyer. It is not difficult to identify the male part of questionable tumors, since in a very early phase of differentiation, the arrhenoblastoma already shows the characteristic polyhedral Leydig cells which surround the trabeculae, long before they become canalized. The female part, the granulosa cell tumor component, offers much greater difficulties for diagnosis. It is a well known fact that in the first immature phases granulosa cell tumor and arrhenoblastoma cannot be differentiated from each other. They both present a very cellular mesenchyme which duplicates the earliest embryonic phases of the gonad and looks like a very cellular fibroma. It is sometimes called sarcomatoid, although it is immature only, and by no means malignant. Similarly as, during the first few weeks of fetal life, ovary and testicle cannot be differentiated from each other, it is also impossible to distinguish the initial immature phases of the corresponding tumors from each other. Only by adjacent parts of high maturity, can immature parts be identified as granulosa cell tumor or as arrhenoblastoma. It is an error that can easily be made, but should be avoided, to diagnose the immature part of an arrhenoblastoma as granulosa cell tumor and to classify the tumor as gynandroblastoma. To make a safe diagnosis of the female component we either have to prove the presence

of full-fledged granulosa cell tumor tissue, which is easy if mature tissue is present, or we have to make the female character of immature parts probable by hormone determination of the tumor tissue or by the effect of these female hormones on the uterus of the patient. Among the dozen cases that Plate reviewed, only his own case, one of my cases (no. 41) and the case of Frankl (published without illustrations) correspond to Robert Meyer's definition; all other cases are either arrhenoblastomas with immature areas (for instance, the case of Eerland and Vos), or atypical granulosa cell tumors (as the case of Amati). In addition to these three cases and Black's case, a fifth case was discovered, but not yet published, by Dr. Christopoulos of Detroit. One of the cases published by M. R. Robinson (*Surg., Gynec. & Obst.*, 1930, **51**, 321, case 1) is suggestive as belonging to this group: a woman, 30 years old, who, during her first pregnancy, terminated in the seventh month of gestation, showed signs of masculinization, such as growth of hair on the body and face and lowering of the voice. The right ovary, which was transformed into a tumor the size of a hen's egg, was removed. The description and four illustrations point to a granulosa cell tumor, but are not sufficient to prove the presence of arrhenoblastoma tissue as suggested by the history.

INTERSTITIAL CELL TUMORS OF TESTES IN MICE INDUCED WITH STILBESTROL. Michael B. Shimkin (by invitation) and Hugh G. Grady, Bethesda, Md. and Darby, Pa.

Abstract. Subcutaneous implantation of pellets of stilbestrol in cholesterol produces a marked hyperplasia of the interstitial cells of the testes in mice of strain C. In 6 to 12 months, about one-fourth of the animals develop frank testicular tumors, some of which metastasize. Testicular tumors of the interstitial cell type are also induced in mice of strains A and c^e, but not in mice of strains C_{57} Black, I, or C_3H. Foster nursing exerts no influence on the appearance of testicular tumors.

Slides were presented of the gross and histologic appearance of the induced testicular tumors in mice.

Discussion

(Dr. Emmerich von Haam, Columbus, O.) Do the tumors regress if the pellet of stilbestrol is removed?

(Dr. Robert C. Grauer, Pittsburgh, Pa.) I would like to ask what the age of these mice was when the pellets were implanted under the skin.

(Dr. Walter Schiller, Chicago, Ill.) I want to ask Dr. Shimkin whether the artificially produced interstitial tumors of the mouse testicle contain hormones, and if so, which hormones. I have had the opportunity to study slides of these tumors and the similarity with some of the human interstitial testicular tumors, for instance, with the case of Masson, is striking. However, the human interstitial tumors, if analyzed from the endocrine point of view, are not a homogenous entity. The majority of the cases found in adult men produce no endocrine changes in the patients. Three cases—those of Stuart, Bell and Roehlke; Rowland and Nicholson, and Sachi—provoked precocious puberty in small boys of 5, 6 and 9½ years. It fits into the conception of a tumor developing from cells which produce male hormones, that these growths provoke precocious puberty in small boys, analogous to the frequently observed precocious puberty with granulosa cell tumors in small girls. In an adult male organism the male hormone cannot provoke any endocrine changes. But there is a group of three cases, those of Hunt and Budd, of Huffman and of Monaschkin, in which interstitial tumors of the testicle produced demasculinization and effeminization of the patient with marked gynecomastia, twice in adults, once in a small boy. This does not fit in the conception of a tumor developing from the endocrine elements of the male gonad. The tumor of Huffman with its abundance of connective tissue and marked tendency for

calcification differs morphologically from the ordinary type. The conception may be raised whether these three effeminizing tumors may be hypernephromas. The effeminizing potency of some adrenal hypernephromas in adult man is well known and remnants of adrepal cortex have been found in the hilum of the testicle in a few cases in the human and are not too rare in certain animals, as in the rabbit. Hormone determinations in artificially produced interstitial tumors in animals and in clinically diagnosed tumors in humans may help to clarify this problem.

(Dr. Shimkin) I do not know whether these tumors regress if the pellet is removed. The pellet was permitted to remain in place in all these animals. In no case did regression occur under these circumstances. The pellets were usually implanted when the mice were 2 to 3 months of age. However, we have implanted them when the animals were barely mature (28 days) to 8 months of age, without any definite difference in effect.

Concerning the hormones, if any, secreted or stimulated by the tumors, I do not know. We were trying to get a sufficient amount of tumor tissue from the transplants for the studies since one cannot get enough from the original tumors. Gardner believes these tumors do have a hormonal effect, but our evidence is inconclusive.

THE INFLUENCE OF THE THYMUS GLAND ON NEUROMUSCULAR FUNCTION IN MYASTHENIA GRAVIS. A. M. Harvey, J. A. Lilienthal, Jr., and S. A. Talbot (by invitation), Nashville, Tenn.

Abstract will appear in the Proceedings of the American Society for Clinical Investigation, published in *The Journal of Clinical Investigation*. The full paper will appear in the September, 1942, issue of that journal.

Discussion

(Dr. Seaton Sailer, Cincinnati, O.) What were the histological changes in the thymus tissue removed?

(Dr. Harvey) None of the patients had a localized tumor of the thymus; there was a generalized hyperplasia of the small round cells of the thymus gland. There were no changes in the epithelial cells in these cases.

(Dr. William H. Harris, New Orleans, La.) I should like to ask if there was any enlargement of other discernible lymphoid structures.

(Dr. Harvey) In none of the cases was there any palpable enlargement of the lymph glands.

FIBROUS OSTEODYSTROPHY ASSOCIATED WITH PIGMENTATION OF THE SKIN. Robert C. Grauer, Pittsburgh, Pa.

Abstract. Differentiation of fibrous osteodystrophy in which there is an absence of demonstrable parathyroid overactivity must be made from those forms in which there is a disturbance in the parathyroid gland. A specific entity in which the osseous disturbance is associated with congenital pigmentation of the skin was described. Two cases, one in an adult female who had been studied over a period of 20 years, and the other in a male child, were presented. Complete studies, which included determinations of calcium balance, hormonal assays, repeated roentgenological examinations and multiple biopsies of various portions of the skeleton, were performed. Subsequent parathyroid exploratory operation was done. The patients are often indistinguishable from those cases showing parathyroid adenomata. The chief characteristics of the condition are a disseminated cystic osteodystrophy; areas of melanotic cutaneous pigmentation in the regions of the greatest bony disturbance; positive calcium balance and evidence of alteration in bone growth as influenced by the endocrins.

The implications in these cases are that we are dealing with an obscure progressive disturbance of bone which has its inception in interference with those elements that govern proper somatic development. The cutaneous and osseous disturbance would ordinarily have its origin in an alteration in the function of the pituitary gland or possibly of the hypothalamus. The etiological factors are uncertain. Recognition of these cases is important in order that they may be differentiated from those amenable to cure through surgical removal of a parathyroid tumor.

Discussion

(Dr. M. Pinson Neal, Columbia, Mo.) May I ask the essayist if there was a disturbance in the hematological picture in these cases?

(Dr. Grauer) Do you mean in the blood counts? If so, there was none.

TRANSPLANTATION OF CHROMOPHOBE ADENOMA-LIKE LESIONS OF THE RAT HYPOPHYSIS. John A. Saxton, Jr., New York, N. Y.

Abstract. Chromophobe adenoma-like lesions of the hypophysis occur frequently at an advanced age in albino rats of the Yale strain. Homologous intra-ocular transplants were made with adenoma-like tissue from two spontaneous lesions, and tissue from one of these is now in the third serial intra-ocular generation. Growth of the transplants is extremely slow, and it has required from 12 to 19 months for the transplants to reach a size suitable for serial transfer. There is usually a latent period of several months before growth can be observed, although the transplants appear to be vascularized within a few weeks. The growth rate has not been accelerated in the second serial transfer, and the morphology has not changed. Although the adenoma-like lesions develop spontaneously only at an advanced age, the age of the recipient has been found not to be a factor either in percentage of successful transplants or in the rate of growth of transplants.

It is concluded that chromophobe adenoma-like lesions of the rat hypophysis behave on transplantation like neoplasms in that their growth rate, while characteristically slow, is independent of the growth rate of tissues of the recipients and in that the ability of transplants to grow is independent of factors associated with aging of the recipient.

Discussion

(Dr. J. M. Ravid, New York, N. Y.) I should like to ask Dr. Saxton whether he studied the relative proportions of the chromophilic and chromophobic cells in the remaining part of the pituitary; also whether he can tell us something about the growth of implanted normal pituitary tissue in the eye.

(Dr. Saxton) The relative proportions of chromophilic and chromophobic cells in other parts of the pituitary were not studied. The general impression was that there was no conspicuous change. As for the growth of normal pituitary tissue in the eye, I believe most studies have shown that normal tissue may persist under these conditions, but that it will not grow, and probably could not be carried through more than one generation.

FUNCTIONING TUMORS OF ENDOCRINE GLANDS. Howard T. Karsner,* Cleveland, O.

Abstract. Certain of the tumors which arise in ductless glands are associated with other bodily changes which can only be explained by the production of hormones by the tumors. The distinction between hyperplasia and neoplasia presents the same difficulties as in the exocrine glands. What are clearly hyperplasias may have the same hormonal activities as the tumors and this feature is of no aid in making the distinction.

* By invitation of the Council.

The tumors may be benign or malignant, but the microscopic criteria usually characteristic in tumors of exocrine glands may be misleading in the tumors of endocrine glands. Thus tumors which are obviously benign may show marked cellular pleomorphism, alteration of polarity and many mitoses. The malignant forms often show these changes, but the alterations may be inconspicuous in the original tumor and its metastases. A diagnosis of malignancy rests on the establishment of invasion, or metastasis, or both. Recurrence, as a sign of malignancy, is undependable, because aberrant rests of glands or their anlagen may give rise to new tumors independently of the original neoplasm.

Regardless of the embryonal origin of the glands, the terms adenoma and carcinoma are usually applied and have obtained good usage. Nevertheless, certain tumors of the ovary, those of the adrenal cortex and perhaps of other situations, are ultimately derived from mesoblast.

The association of special functional changes with tinctorial and other properties of the neoplastic cells does not seem to be wholly justified. There are certain variations from the expected in acidophilic and basophilic lesions of the pituitary. The functioning islet cell tumors of the pancreas have not been shown to possess any unusual numbers of beta cells. There is no clearly established relationship between any particular cell of the adrenal cortex and the changes in secondary sex characters. The lipid-containing cells sometimes seen in arrhenoblastomas of the ovary are not necessarily homologues of the interstitial cells of the testis.

It is usually assumed that hormones are produced because of the somatic effects of the tumors. In only a few of those associated with gonads and adrenals has there been any satisfactory assay of hormones and such assays are practically limited to the steroidal sex hormones. The influence of intermediate products and metabolites is not yet fully established, nor is the influence of quantities and proportions altogether clear. Further studies should be made at every opportunity and may aid not only in explaining the activity of the tumors but also in the final identification of some of those whose nature is not yet established.

A Case of Paget's Disease of Bone with Autopsy Report. James Miller, Kingston, Ontario.

Abstract. The patient, E. S., was born in the south of England in 1870. She was a widow and previous to marriage worked as a weaver. She was a member of a family of six; four girls and two boys. There is no record that either of the parents suffered from bone disease, but all four sisters have developed symptoms suggestive of Paget's disease and one, who is resident in Canada, has distortion of most of the bones of the body and has had repeated spontaneous fractures. There are no precise data of the onset of the disease in the patient but it was apparently well marked in 1928. She was admitted to Rockwood Mental Hospital in 1940 and died in March, 1942. The diagnosis in her case was senile psychosis with simple deterioration. Clinical pathological observations carried out during hospitalization were as follows: serum calcium, 10.2 mg.; blood urea, 24 mg.; hemoglobin, 60 per cent; red blood cells, 2,580,000; leukocytes, 5,760. The Wassermann test was negative.

The relevant autopsy findings were as follows: The calvarium was enormously enlarged, measuring 63.5 cm. in circumference. When removed, it weighed 1740 gm. It varied in thickness from 1 to 3 cm. The distinction between diploë and outer and inner tables had disappeared, the whole bone being transformed into soft osteoid material red in color and easily cut with the saw, and some parts with the knife. The spine showed well marked kyphosis in the dorsal region. The long bones, radius, ulna, femur and tibia, were bent, thickened and softened, and the marrow of the shafts was red and partially occupied by soft bone. The stomach

showed a large ulcerating carcinoma in the region of the pylorus and there was a small rounded meningioma attached to the dura mater over the left frontal area 3 cm. from the middle line. There was atheromatous change in the aorta and the coronaries, and the arteries generally, especially those of the lower limbs, were extensively calcified. In regard to the endocrine glands, the adrenals were large but normal and the pituitary was flattened, as was also the brain, by a projection upwards of the base of the skull; the thyroid was small and showed nothing of note microscopically; the parathyroids were dissected out and found to be normal.

Microscopically the bony changes were in conformity with those usually found in Paget's disease, the only unusual feature being well marked calcification of the media of the arterioles in the substance of the bone itself.

Discussion

(Dr. W. E. B. Hall, Port Huron, Mich.) I should like to ask Dr. Miller if, in the examination of the bones, he found any areas showing giant cell formation, and also if he found any areas in which considerable hyperplasia of the osteogenic elements was present, such as we might expect to find preceding the formation of forms of osteogenic sarcoma.

(Dr. Miller) There is one point which I should have mentioned: tumors are extremely frequent in these cases, and this patient had a carcinoma of the stomach and a meningioma—a typical psammoma of the dura mater—but she had no tumors of the giant cell type, and nothing to suggest osteogenic sarcoma.

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RELATIONSHIP OF NEUROFIBROMATOSIS OF THE SKELETON TO PSEUDO-ARTHROSIS. William T. Green and Nathan Rudo (by invitation), Boston, Mass. Presented by Ruell A. Sloan.

Abstract. Although pseudo-arthrosis and "congenital bowleg" have been described as skeletal changes which may occur with neurofibromatosis, the mechanism by which pseudo-arthrosis arises has not been explained, nor are we able to find a pathologic description of tissue from the area of pseudo-arthrosis.

A patient, a white female, 7 years of age, was first seen complaining of a tender mass in her right arm of 8 months' duration, and a bowing of the right leg which had been present since birth. Pertinent physical and roentgenographic findings at this time included café-au-lait spots on the skin, a tumor mass of the right arm which was demonstrated microscopically to be a plexiform neuroma, an anterolateral bowing of the tibia with increased density of bone and a pseudo-arthrosis of the fibula. In the patient's subsequent course, a localized tumor developed in the tibia, and two pathologic fractures occurred in this bone. One of these has not united despite surgical intervention.

Histologic examination of tissue from the pseudo-arthrosis of the fibula, from the tumor defect in the tibia and from the pathologic fractures of the tibia all revealed typical neurofibroma. In this case, the pseudo-arthrosis seemed to be associated directly with intra-osseous neurofibromata which had caused pathologic fractures of the tibia and fibula and retarded or prevented bony union.

In all cases of so-called congenital pseudo-arthrosis, the possibility that neurofibroma is the etiologic factor must be entertained. Tissue removed from the area of the pseudo-arthrosis at operation should be scrutinized carefully in an attempt to demonstrate or exclude the possibility of neurofibroma, realizing that superficial examination may suggest the diagnosis of "scar tissue." It should be emphasized that it may be difficult to differentiate neurofibroma from fibrous tissue of other origins. If neurofibroma is present, incomplete excision of the tissue may lead to recurrence and be a factor in non-union.

Discussion

(Dr. Edward A. Gall, Cincinnati, O.) I wonder if the authors have any explanation for the peculiar sigmoid deformity of the tibia.

(Dr. Sloan) The cause of the bowing of the tibia is not readily apparent. We have seen several other patients with a bowing of similar character in association with neurofibromatosis. The discovery of tumor tissue in the shaft of the bone might lead one to speculate as to the possibility of the presence of neurofibroma at the zone of enchondral ossification, causing inequality of the rate of bone growth and consequent deformity. Under such circumstances, with progression of the growth zone away from the tumor, the normal rate of growth might be re-established. No evidence to support this theory could be determined, as roentgenographically no definite defects in the tibia were demonstrated before the child was admitted to the hospital at the age of 7 years.

(Dr. Robert C. Grauer, Pittsburgh, Pa.) Was the rest of the skeleton x-rayed in order to determine whether any other defects were present by roentgenologic evidence?

(Dr. Sloan) Skeletal survey showed the remainder of the skeleton to be free of lesions.

VISCERAL LESIONS ASSOCIATED WITH CHRONIC INFECTIOUS (RHEUMATOID) ARTHRITIS. A. H. Baggenstoss and (by invitation) E. F. Rosenberg, Rochester, Minn.

Abstract. Many investigators feel that rheumatoid arthritis is a generalized systemic disease and that arthritis is merely one of its manifestations. The present study was undertaken primarily to determine the nature of the anatomic changes which occur in the viscera in rheumatoid arthritis. It was based on a study of the necropsies in 30 cases of chronic infectious (rheumatoid) arthritis. There were 17 males and 13 females. The mean age of these patients at the time of death was 38 years. The youngest patient was 9 years and the oldest 81 years of age. Only 5 patients were 60 years old or over.

Cardiac lesions were present in 24 cases. Lesions indistinguishable from those of rheumatic fever were observed in 16 cases, or 53 per cent. Non-rheumatic lesions were present in 8 cases (27 per cent). In 9 of the 16 cases which had rheumatic cardiac lesions the heart disease was judged to be an important factor in causing death. The lungs were frequently the site of infectious processes in patients with rheumatoid arthritis but it is difficult to consider these as being anything more than coincidental. Many of the pulmonary infections were, in fact, terminal. A slight degree of splenic enlargement was common. The mean weight was 250 gm. The spleen weighed over 300 gm. in 9 cases. In most of these cases the enlargement was found, histologically, to be on the basis of either chronic passive congestion or reticulo-endothelial hyperplasia. Amyloid disease occurred in 2 cases. Chronic passive congestion of the liver was present in 18 cases, fatty change in 7 cases. Atrophy of the hepatic parenchyma was present in practically all of the cases which had passive congestion. Actual necrosis of the hepatic cells occurred in 4 cases. Inflammatory lesion occurred in the intestinal tract in 4 cases, in the pancreas in 3 cases, in the prostate in 2 cases and in the adrenal gland in 2 cases. Subacute suppurative interstitial nephritis occurred in 3 cases, and non-suppurative pyelonephritis occurred in 1 case. Proliferation of the endothelial cells of the glomerular capillaries (glomerulitis) was present in 19 cases (63 per cent). Nine of the 19 cases (47 per cent) with glomerulitis had evidence of either active or healed endocarditis. It may be that in these cases the renal lesion was a result of the endocarditis, but we feel that it is more reasonable to attribute both cardiac and renal lesions to the same underlying disease.

The character of the lesions in the heart, kidneys and other organs in these cases gives further support to the view that rheumatoid arthritis is in all probability an infectious disease. The nature of this infection is not clear from this study, but the high incidence of rheumatic cardiac lesions in this series is suggestive of a relationship between chronic infectious arthritis and rheumatic fever.

Discussion

(Dr. Jacob Werne, New York, N. Y.) I am very much interested in the lesions, other than those rheumatic in character, which Dr. Baggenstoss described. I have encountered glomerular changes of the type described, and foci of cellular infiltration in visceral sections in my series of infants dying unexpectedly of fulminating infection. May they not be considered as manifestations of the acute infections which brought these cases to autopsy, rather than as lesions that are specifically part of the chronic infectious arthritic disease? To phrase the matter differently, I wonder whether these same lesions would be found in cases dying of intercurrent violence rather than of their infectious arthritis.

(Dr. Baggenstoss) Of course it is impossible to state in every instance whether the lesions described were the result of a terminal infection or whether they were an integral part of the disease known as rheumatoid arthritis. I think there are two things to bear in mind. Almost all of these patients came to the clinic with an active rheumatoid arthritis. The joint disease was not quiescent; the patients had an active arthritis and came for the treatment of this condition. Another thing to bear in mind from Dr. Bell's study on glomerulitis is that in patients dying with lobar pneumonia, only about 18 per cent have a glomerulitis and yet in the present group of cases 63 per cent had glomerulitis. Terminal pulmonary infections would not account for such a high incidence.

CHEMICAL ISOLATION WITH PRESERVATION OF STRUCTURE AND FUNCTION OF ELASTIC TISSUE OF THE HUMAN AORTA. George Hass, New York, N. Y.

Abstract. This report was a presentation of a method for dissolving all components of the fresh human aorta except elastic tissue. The elastic network with all preexisting structural continuities and staining properties are preserved in such perfect state that a pure tissue becomes available for correlative physical, morphological and chemical studies. The pure tissue is more elastic in the common sense than the aorta from which it is obtained and displays various other properties which cannot be estimated by studies of the intact aorta.

A FATAL DISEASE OF MIDDLE-AGED MICE WITH MYOCARDITIS ASSOCIATED WITH HEMORRHAGE IN THE PLEURAL CAVITY.* D. Murray Angevine and Jacob Furth, Wilmington, Del. and New York, N. Y.

Abstract. A disease complex hitherto not described is occurring in small numbers of all stocks of normal mice in an animal colony comprised of from 5,000 to 7,000 mice per annum. It is found in about 1.2 per cent of all mice dying spontaneously and is characterized by myocarditis with sudden death, usually from hemorrhage into the pleural cavity. The hemorrhage is due to rupture near the origin of the great vessels. Gross or microscopic testicular hemorrhage was usually present. Hyaline degeneration of blood vessels in the lungs and testes was frequently observed. The disease affects male mice with rare exception. It is most common between 10 and 19 months with a peak at 14 months. Healthy mice are apparently affected and they appear normal until death occurs.

Transmission experiments to normal mice have been unsuccessful and cultures failed to yield an organism that could be regarded as causing the disease. Epidemiological observations likewise did not indicate an infectious disease.

* Accepted for publication in the *American Journal of Pathology.*

Discussion

(Dr. Jacob Werne, New York, N. Y.) I would like to ask Dr. Angevine whether he did any bacterial stains on his visceral sections. I would also ask why he considered the *Staphylococcus aureus* as a contamination. In my human material I have occasionally found acute myocarditis in persons dying of fulminating infection. In several instances that I recall, *Staph. aureus,* probably entering through the upper respiratory tract, was the offending organism.

(Dr. Stuart Lippincott, Bethesda, Md.) Occasionally we see myocardial lesions in strains of mice such as the C_3H strain, and usually they are preceded by degeneration, followed by calcification, but there is no other lesion in the body. I wonder whether in the old mice of this colony you ever see calcification.

(Dr. Angevine) In answer to Dr. Werne's question, we made many bacterial stains on the viscera of these animals, and found nothing significant. If I said *Staph. aureus,* it was a mistake, for I meant *Staphylococcus albus.*

The majority of mice of this colony died before 12 months, so that we did not have many animals reaching old age. However, calcification was not observed in the hearts of the older mice examined.

LOCAL ORIGIN IN HUMAN EXTRAMEDULLARY MYELOPOIESIS. Walter Schiller, Chicago, Ill.

Abstract. Two explanations have to be considered for the development of extramedullary myelopoiesis. The first is the assumption of local origin. This theory meets with greater probability when the organs in question are those normally concerned with hematopoiesis in fetal life, as liver and spleen. With anemias the presence of erythropoietic tissue in these organs may be interpreted as a compensatory revival of a prior function which physiologically is not carried on in extra-uterine life. A subgroup of the theory of local myelopoiesis which finds good support in many animal experiments concerns the transformation of vascular endothelium (Dieckmann), of perivascular adventitial cells (Bloom, Herzog), or of undifferentiated mesenchymatous elements mostly located in the vicinity of blood vessels (Jaffé, Lang), into undifferentiated lymphoid cells or hemocytoblasts, after irritation or stimulation by injection of dead bacteria, as proteus or coli; of poisons, as saprotoxin, or pyrogallol, or after inoculation with transplantable tumors. The second theory traces the extramedullary foci of myeloic tissue back to hematogenous immigration of immature bone marrow cells which enter the circulation in the bone marrow. This process, which is analogous to the metastasizing of malignant tumor cells by the blood stream, has been called colonization by some authors, a term coined to use instead of "metastasizing."

Local origin is much the more difficult to prove. Two ways are open. The first is to find evidence of local transformation of mesenchymatous cells, particularly of cells of the reticulo-endothelial system into immature blood cells; that is, metaplasia *in situ.* The second way is to find a case which shows abundance of the immature blood elements in the bone marrow and in well developed extramedullary foci but with the circulating blood free from such cells. Quantitative differences between the incidence of immature blood cells in the blood and in the tissue do not prove much, since even a few intravascular cells may give origin to extensive metastases as is observed in malignant tumors. Only qualitative differences are convincing and particularly the absence of the immature elements in the blood, which are present in the tissues. After unsuccessful examination of numerous cases of acute and chronic lymphatic and myelogenous leukemia, a colored woman, 46 years old, with chronic myelogenous leukemia was found who expired when the neutrophilic leukemia was on the verge of changing into an eosinophilic one. Whereas the bone marrow and the liver at autopsy contained huge numbers of

eosinophilic promyelocytes and myelocytes, only the last blood examination a short time before death showed a low percentage of eosinophilic metamyelocytes and polymorphonuclears. Slides of the liver gave evidence of a gradual transformation of Kupffer cells into eosinophilic myelocytes by the development of eosinophilic granules and retraction of the stellate projections of protoplasm. After this metaplastic change, the cells entered the blood of the sinusoids as eosinophilic granulocytes.

UNRELIABILITY OF BLOOD FINDINGS AS CRITERIA OF SEVERITY OF SHOCK IN RABBITS. Milton D. Bosse (by invitation) and Paul Gross, Pittsburgh, Pa.

Abstract. Seventy rabbits were burned by partial immersion in hot water under ether anesthesia. Although in the majority of animals varying degrees of hemoconcentration and hypoproteinemia were demonstrable in addition to clinical manifestations of shock, a number of notable exceptions were encountered. It was found that in a number of animals which died of shock, the hemoconcentration was quickly followed by hemodilution, and in several the picture of shock was associated with hemodilution from the onset. The degree of hemoconcentration was not an accurate indication of the degree of shock because many of the animals that died exhibited a relatively mild hemoconcentration. In some rabbits dying of shock the blood protein had returned to the normal level, or above, at the time of death. These exceptions do not conform to results obtained with other animals and cannot be explained at the present time.

Discussion

(Dr. Virgil H. Moon, Philadelphia, Pa.) I am interested in this investigation, and I can support the essayists' findings of hypoproteinemia after burns and incident to shock from other causes. However, I should like to ask the essayists if they have used other animals than rabbits in any of their experiments. We have made various experiments on different types of animals and have found results more variable in rabbits than in any other species. Some of our early experiments on shock were made on rabbits. These showed more individual variation than did other animals; accordingly, our subsequent experiments were made on dogs in preference to rabbits. Did you make your experiments on any other animals?

(Dr. Bosse) These experiments are reported because, if the present theories of shock are correct, they should apply to all animals which manifest shock. We worked only with the rabbit in the experiments and the results obtained do not conform.

(Dr. Moon) That coincides with our experience. I should like to ask whether the hemoconcentration was observed by means of the determination of the specific gravity of the blood, by hemoglobin and red cell counts, or by the hematocrit.

(Dr. Bosse) The plasma protein was determined by the micro-Kjeldahl method. The hemoconcentration was determined by the red blood cell count, although in most cases hematocrit readings were also made and the changes corresponded very closely to those in the red blood cell counts.

(Dr. Moon) We have come to the conclusion that these determinations are best made by the red blood cell count. I think this is more accurate for determining hemoconcentration than is the specific gravity of the blood.

HEMATIC AND ORGANIC CHANGES AFTER INTRAVENOUS ADMINISTRATION OF PECTIN SOLUTIONS. W. C. Hueper, New York, N. Y.

Abstract. Rabbits and dogs were injected with neutralized 1 and 2 per cent colloidal pectin solutions over periods of several months. The immediate hematic effects consist in a colloidoclastic leukopenia lasting for several hours and a hastened

erythrocytic sedimentation lasting for more than a week. The hematic effects of repeated injections consist mainly in an accelerated sedimentation rate. Pectin is stored in the liver, spleen, kidney and bone marrow, giving rise to a foam-cellular transformation of the reticulo-endothelial cells of these organs as well as of the liver cells. Giant-cellular granulomas are found in the pulmonary arterioles. Atheromatous lesions of extensive degree and medial degeneration and calcification are elicited by pectin in the aorta and in large and small arteries. Thus, additional evidence is advanced in support of the thesis that macromolecular colloidal disturbances of the blood plasma are causally related to the development of arterial atheromatosis and organic thesaurosis.

Discussion

(Dr. Hans Popper, Chicago, Ill.) I would like to ask Dr. Hueper the following questions: First, how did the dosage of pectin he used compare with the doses Hartman and his associates used as blood substitutes in man? Second, did he use autoclaved pectin solutions as Hartman did and, if so, what results he got with them? Third, how long did the storage of the material in the organ last?

(Dr. Hueper) Our dosage cannot be compared with the dosage of the Hartman experiments, because this investigator used autoclaved pectin solutions. When a pectin solution is subjected to autoclaving, a heavy precipitate is formed, which is especially marked if the solution was neutralized before being subjected to heat. This precipitate is filtered off and thus a severe loss of pectin is sustained. In addition to this reduction in concentration of pectin, an autoclaved solution contains also a pectin of considerably decreased molecular weight. The heat degradation of pectin manifests itself in a considerable lowering of the degree of viscosity. Bryant has prepared a chart in which the depolymerization of the pectin by hydrolysis under the influence of autoclaving is demonstrated. He found that a pectin solution consisting originally of molecules having a molecular weight somewhat above 200,000 was composed, after being autoclaved for 30 minutes at 15 pounds pressure, of molecules of an average molecular weight of about 50,000. In my experiments with autoclaved pectin solution there was little retention of the degradation product in the internal organs. In some of the animals foam cells were found in the bone marrow. It is my opinion that pectin solutions are unsuitable for the treatment of shock, as they cannot be well standardized as to concentration and physicochemical properties when subjected to autoclaving and are highly unstable even when kept in the ice box.

HISTOGENESIS OF GASTRIC ULCERS IN RATS PRODUCED BY FASTING AND PARTIAL INANITION. Stuart W. Lippincott and (by invitation) Harold P. Morris, Bethesda, Md.

Abstract. The production of gastric lesions in rats by several experimental procedures has attracted the attention of many investigators during the last quarter of a century. The etiologic factors presumably involved have varied considerably. Of significant interest has been the purported relation of certain dietary deficiencies to the genesis of ulcers of the glandular portion of the stomach. Our investigations upon 14 experimental groups of rats showed that superficial ulcers of this region developed during fasting and partial inanition, but never were observed during the individual absence from the diet of fat, carbohydrate, or protein.

It was observed microscopically that a circulatory disturbance initiated the changes resulting in the formation of the mucosal ulcers of the glandular portion. The peripheral capillaries, in focal areas, became hyperemic, followed by escape of red blood cells. The spread of the red blood cells was restricted at first to the relatively small amount of loose connective tissues lying between the intact acini.

During this period the parietal and chief cells of the acini appeared normal, and the neck cells were still filled with mucus. As stasis and hemorrhage increased, mantles of red blood cells became confluent. At this time many of the mucus-secreting neck cells disappeared, but some of them persisted. Simultaneously the acini began to lose their normal cellular arrangement. The lumina of acini disappeared as the margins of the cells became indistinct. Nuclei of adjacent cells became pyknotic. Shallow ulcers formed as the result of whole groups of acini becoming necrotic, together with the destruction of the overlying layer of mucous cells. In sections stained by hematoxylin and eosin the ulcers often appeared yellowish green, and this suggested bile deposition. However, with Stein's technic bilirubin was not found. With the loss of overlying necrotic tissue, the margins and bases of these ulcers became clearly defined. Inflammatory exudate was absent. The ulcers were usually limited to the outer half of the mucosa but occasionally extended down to the muscularis mucosae. It is apparent that the process did not originate with a primary local loss of continuity in the layer of mucus-secreting cells. Instead, there was a local circulatory disturbance with subsequent interference in the nutrition of all the cells of that region which resulted in necrosis of acini and the formation of superficial mucosal ulcers.

Discussion

(Dr. Carl V. Weller, Ann Arbor, Mich.) I noted with interest reference to the rather heavy eosinophilic infiltration. We were asked recently to examine the alimentary tracts of rats used by Professor Norman Maier of the Department of Psychology of the University of Michigan, in the production of neurosis and psychoneurosis in rats by thwarting conditioned reflexes. One of our internists thought those rats should have ulcers after such an experience, and we were asked to examine them. I found very heavy eosinophilic infiltration in most of them, and no active ulcers. A very small percentage showed acute erosions which might be beginning ulcers. The only explanation which I found for the eosinophilia was the presence of nematodes in considerable numbers in the intestinal tract. I mention this without any disparagement of the authors' results, but I think the presence of eosinophils should be interpreted with caution.

HISTOGENESIS AND REPAIR OF HEPATIC CIRRHOSIS IN RATS PRODUCED ON LOW PROTEIN DIETS AND PREVENTABLE WITH CHOLINE. R. D. Lillie, L. L. Ashburn and (by invitation) W. H. Sebrell, F. S. Daft and J. V. Lowry, Bethesda, Md.

Abstract. The hepatic cirrhosis of rats produced by Lillie, Daft and Sebrell on low protein, choline-deficient diets is characterized by centrolobular fatty changes, soon followed by the appearance of an acid-fast sudanophilic hyaline material, designated as ceroid, in liver cells and interstitial, subcapsular and centrolobular phagocytes. Soon after this trabeculae of ceroid phagocytes in fibrous tissue form. Choline treatment removes the fat from the liver cells and increases their size, but leaves untouched the fibrous trabeculae and ceroid phagocytes. This cirrhosis differs from any we have seen in man and from other experimental cirrhoses.

VARIATIONS IN THE DISTRIBUTION OF VITAMIN A IN THE HUMAN LIVER UNDER PATHOLOGIC CONDITIONS. Hans Popper, Chicago, Ill.

Abstract. Various pathologic conditions of the liver reveal under the fluorescence microscope characteristic variations in the amount and distribution of vitamin A. These changes (in human liver) are frequently more characteristic than those found with routine histology. Animal experiments with various diets and intoxications aided in recognizing the significance of some of the typical changes in the pathologic human liver. An inability of the damaged epithelial cells to hold or discharge

vitamin A; a block between Kupffer and epithelial cells in its transmission, and an inability of the Kupffer cells to absorb vitamin A from the blood or to split carotene were demonstrated. Conclusions as to vitamin A therapy were drawn.

THE INCIDENCE OF RHEUMATIC STIGMAS IN NON-RHEUMATIC HEARTS. Ernest M. Hall and (by invitation) Lucille R. Anderson, Los Angeles, Calif.

Abstract. In a previous study an attempt to find normal hearts that showed no rheumatic stigmas when examined according to the standard method of Gross, Antopol and Sacks was unsuccessful. It seemed worth while to investigate the matter further. The authors have studied 124 hearts; 12 of these revealed evident lesions of rheumatic or bacterial endocarditis and were used as controls. The remaining 112 hearts were free of evident gross lesions of the valves although many revealed minimal thickening of valve edges. Some of the hearts in the larger group were sectioned according to the standard methods. In the majority blocks were sectioned from only two areas: (1) through the posterior papillary muscle; (2) through the base of the left ventricle including a portion of the posterior mitral leaflet.

The more common stigmas which are usually considered indicative of rheumatic infection were employed; *viz.*, rheumatic arteritis, fibrinoid necrosis and elastic tissue alterations, Aschoff bodies, perivascular fibrinoid swelling or fibrillary scarring, increase in cardiac histiocytes in the region of the vessels and in the interstitial tissue. Sixty-eight, or 60.8 per cent, of the 112 hearts exhibited abundant rheumatic stigmas and were considered "positive" for rheumatic infection. Thirty-six hearts (32.1 per cent) were moderately involved and were called "probable rheumatic infections." Only 8, or 7.1 per cent, were considered doubtful. Aschoff bodies were found in 33 hearts (29.5 per cent) while Aschoff-like nodules consisting of cardiac histiocytes were seen in 34 cases, or 30.3 per cent. Among the 12 control cases there was a history of rheumatic fever in 9 cases. Aschoff bodies were found in 9 of the 12 cases. Of this control group rheumatic stigmas were abundant in 11 and moderate in 1.

Do the results of this study signify a practically universal rheumatic infection in much the same sense as tuberculosis is essentially universal? If so, most persons are relatively immune just as they are to minute quantities of tubercle bacilli. The etiologic agent in rheumatic fever is unknown but much evidence points to the hemolytic streptococci as either directly or indirectly of importance. The rheumatic stigmas found in clinically and anatomically non-rheumatic hearts may represent only hyperergic reactions to recurrent sensitizations with the proteins of hemolytic streptococci. These may occur with attacks of upper respiratory infections. It makes no difference, however, whether the etiologic agent is a streptococcus, a virus, or some other organism, since the principle remains the same.

Discussion

(Dr. Howard T. Karsner, Cleveland, O.) In Cabot's book, "Facts About the Heart," he described incidentally, rather than as a major part of the work, what he calls non-deforming chronic endocarditis. He did not, however, definitely attribute this lesion to rheumatic disease. During the course of the past 2 years we have been watching for non-deforming chronic disease of the valves and our data are in general agreement with the observations of Dr. Hall. Valvular disease of the heart occurs much more frequently than would appear on casual examination. A striking feature is the thickening along the edge of the anterior leaflet of the tricuspid valve, often accompanied by some fusion of the chordae tendineae. The pulmonic cusps are likely to show transverse striae of fibrosis and often a slight deformity along the free border. The aortic cusps often show slight sub-marginal

adhesion in the commissures, which is easily overlooked, and there is often fibrosis along the line of closure or the free border. In the mitral valve, as Dr. Hall described, there is thickening along the free border, sometimes accompanied by adhesions between the leaflets, as well as thickening of the chordae tendineae; vascularization of the anterior leaflet frequently occurs. There is also more or less marked roughening of the posterior wall of the left atrium. In our last 100 consecutive autopsies, gross findings were present in 30 cases. We think of these as non-deforming in the sense that they probably, and almost certainly, produced no functional lesion in the valvular orifice. We think they probably represent a healed lesion in the valves and I believe we agree with Dr. Hall in that respect. If a pathologist refuses to make a diagnosis of valvular disease unless he finds changes sufficiently marked to produce functional disturbance, such as pronounced adhesions of the commissures, or retraction of the cusps and leaflets, he will miss many cases.

We are of the opinion that the early lesion of rheumatic endocarditis may become a progressive chronic inflammation which ultimately leads to marked deformity, or that the lesion may become quiescent to remain in a healed stage for many years with no evidence of stenosis or insufficiency. That these healed lesions are the sequel of rheumatic disease is a problem deserving the careful study given by Dr. Hall. We pay little attention to the history of rheumatic fever, not merely because of the fallibility of histories but also because of the likelihood that rheumatic fever may be sub-clinical. The work of Karsner and Bayless showed the frequency of disease of the coronary arteries in rheumatic disease, but did not prove these changes to be pathognomonic. It may be that some of the valvular disease referred to, quiescent and non-deforming, may be the result of other forms of acute endocarditis, but every available clue points strongly toward rheumatic origin.

(Dr. Hall) I am grateful to Dr. Karsner for the discussion. I am especially glad that he accepts the thickening of these valves as being probably rheumatic. I did not have time to go into it as carefully as he did. I personally performed about 25 per cent of these autopsies.

(Dr. Karsner) Let me interrupt you. I did not perform any of them myself, but I saw the material from all.

(Dr. Hall) We have about 1,100 autopsies a year, and I cannot do them all myself. There were only 3 cases of the 112 studied in which a history of rheumatic fever was recorded. I might say that Karsner and Bayless was my bible in this study.

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A Transplantable Spontaneous Hepatoma in a Strain C_3H Mouse. Jesse E. Edwards and (by invitation) Albert J. Dalton and Howard B. Andervont, Bethesda, Md.

Abstract. A primary spontaneous hepatoma in a male C_3H mouse, 17 months old, of the Andervont line has been successfully transplanted for 4 generations in the subcutaneous tissues of mice belonging to the homologous strain and line. Grossly, the primary tumor was a globular, discrete, pale yellow mass 1.2 cm. in diameter. Microscopic examination showed it to consist of liver-like cells with faintly basophilic cytoplasm and well-defined cytoplasmic membranes arranged in obvious cords alternating with endothelial-lined blood sinuses of varying width. No pigment was demonstrated. The tumor was not encapsulated and compressed the adjacent hepatic tissue. The neoplasm could be distinguished from liver by the relatively large cells with basophilic cytoplasm, by the absence of a definite lobular pattern and the absence of bile ducts, except for a few distributed haphazardly in peripheral areas. There were no metastases.

The transplants, composed of soft, friable, pinkish yellow tissue, appeared similar to the primary tumor, except for a greater number of mitotic figures and absence of any bile ducts. Special stains revealed abundant glycogen and neutral fat in the cytoplasm of the tumor cells. Alkaline phosphatase was absent. Significant differences from normal liver cells have been noted in the Golgi apparatus and in the mitochondria of the tumor cells. In normal hepatic cells the mitochondria vary from short, plump rods and spheres at the periphery to fine, tenuous filaments at the center of the lobule. The mitochondria present in transplants of this tumor were practically all small spheres. The Golgi apparatus of normal liver cells always shows a peripheral distribution near the bile capillary, while that of the tumor cells, though large in amount, appeared to be more condensed and formed a mass situated nearer the nucleus.

A transplantable hepatoma arising spontaneously in a strain CBA mouse has been described (Strong, L. C., and Smith, G. M. *Am. J. Cancer,* 1936, **28**, 112–114). To our knowledge, the current case is the first reported transplantable spontaneous hepatoma in a mouse of the C_3H strain. The tumor is being maintained at the National Cancer Institute and is designated as mouse hepatoma 98/15.

INTRATESTICULAR HEMORRHAGE: A BIRTH TRAUMA. Bela Halpert, New Orleans, La.

Abstract. Routine gross and microscopic examination of the testicles of newborn infants at necropsy led to the conclusion that quite extensive intratesticular hemorrhages occur rather frequently as an injury sustained during birth in cases where the head is the presenting part.

SPONTANEOUS CEREBELLAR HEMORRHAGE (REPORT OF FIFTEEN CASES).* Nathan Mitchell and Alfred Angrist (by invitation), Jamaica, N. Y.

Abstract. Fifteen cases of spontaneous cerebellar hemorrhage were encountered in a series of 3,881 autopsies in a 5-year period, during which interval 100 instances of hemorrhage into the cerebrum were noted. The erroneous impression exists that cerebellar hemorrhage is a rare lesion. Only 109 cases of spontaneous cerebellar hemorrhage were found in the literature, and the reported incidence, as compared to cerebral hemorrhage, varies from 0.9 to 10 per cent. It is of interest to note that the ratio of cerebellum to cerebrum by weight is 12+ per cent.

The clinical syndrome of cerebellar hemorrhage is a variable one and this, in part, accounts for the impression of a low incidence. Such patients either die suddenly, particularly when rupture into the ventricular system or subarachnoid space complicates the picture, or they recover. In the former event, it is difficult or impossible to discover at autopsy the exact origin of the hemorrhage. In 15 cases, 6 died suddenly or were found dead, in contrast to only 3 found to have died suddenly in the 109 reported cases. A characteristic neurological syndrome such as occurs in cerebral hemorrhage is rather uncommon. Associated lesions included cardiac hypertrophy, uremia, and leukemia; these are identical with the factors commonly associated with cerebral hemorrhage. Cerebellar hemorrhage represents a lesion comparable to cerebral hemorrhage in incidence and identity of contributory pathological factors, with a tendency to extremes in the clinical picture.

THE OCCURRENCE OF A DOUBLE ZONE PHENOMENON IN ANTI-HUMAN TISSUE SERUM. Anderson Nettleship, Bethesda, Md.

Abstract. Through the use of an optimal precipitin method it was possible to show a double zone phenomenon in duck anti-human kidney serum. This double zone

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apparently is caused by differential chemical structures involved. The higher molecular weight substances—globulins—precipitate in lower dilutions. Those of smaller molecular weight—albumins—combine in higher dilutions.

REDUCTION OF TUBERCULIN REACTIVITY FOLLOWING INHALATION OF FUMES FROM BOILING BACILLI. Sol Roy Rosenthal, Chicago, Ill.

Abstract. In the Tice Laboratory, in which the BCG vaccine (Bacillus of Calmette and Guerin, an avirulent tubercle bacillus) is prepared and administered, it was noted that certain members of the staff would develop periodically symptoms varying from malaise to chills, fever (up to 104° F.), tickling sensation in the posterior pharynx, cough, vomiting, headache and backache. After several months, such responses lessened and finally abated. The possibility that the above symptoms might be related to a generalized tuberculin reaction was suspected for the first time when a new member of the staff who reacted to 0.00001 mg. of O.T. developed a similar syndrome. In tracing the source, it was discovered that the periodicity of the responses was related to the boiling of cultures or vaccine. It was inferred that the vapors might be the offender and in order to test this assumption, animal experimentation was resorted to.

The method employed was to allow fumes from a boiling vessel to enter a sealed box in which guinea pigs were placed. Visible vapors should permeate the enclosure at all times. The time of exposure for animals that reacted to tuberculin (following intraperitoneal injection with BCG) was 1 minute the first day, increasing by 1 minute daily the first week, 2 minutes the second week, and 3 minutes the third week until 60 minutes was reached. The period of exposure to the vapors at one time was never over 15 minutes (to prevent suffocation). Tuberculin testing was performed at regular intervals using old tuberculin and purified protein derivative. In numerous experiments using more than 100 animals, the following observations were made: The cutaneous response to tuberculin could be appreciably reduced in guinea pigs, made reactive by the injection of avirulent tubercle bacilli, following daily exposures to: (1) fumes of boiling suspension of tubercle bacilli; (2) sprays of heat-killed bacilli, and (3) fumes of boiling suspension of tubercle bacilli passed through a fritted glass filter (to trap the bacilli when they issued with the vapors).

THE PATHOGENESIS OF THE LESION ASSOCIATED WITH PANCREATIC ACHYLIA. Sidney Farber, Boston, Mass.

Abstract. A lesion of the pancreas characterized by dilatation of the ducts, inspissation of secretion and connective tissue replacement of atrophied acinar structures is found in constant association with two apparently distinct, clinical pictures in infants: (1) The pancreatic fibrosis variant of the celiac syndrome, and (2) meconium ileus. Evidence is presented to show that the time at which the pancreatic lesion occurs determines whether the clinical picture will be that of the celiac syndrome or of meconium ileus, and that pancreatic achylia secondary to interference with the liberation, formation, or passage into the duodenum of pancreatic enzymes is the defect of fundamental importance in both clinical pictures. Although pancreatic achylia may be produced in these patients by congenital atresia or severe stenosis of the pancreatic ducts, it is unassociated in the vast majority of instances in our experience with any such gross anatomical interference to the outflow of pancreatic juice. These studies do not permit the conclusion that either vitamin A-deficiency disease or a filtrable virus infection is of primary importance in the production of this pancreatic lesion. Although a defect in the absorption of vitamin A is a feature of the pancreatic variant of the celiac syndrome, this pancreatic lesion occurs in the absence of evidence of vitamin A-deficiency disease and persists after the disturbance in absorption of vitamin A has been corrected.

Intranuclear and cytoplasmic inclusion bodies were found in various organs in 12 per cent of 50 patients suffering from this pancreatic disease, an incidence no greater than the average for all autopsies performed in this laboratory. Evidence obtained from postmortem examinations, analysis of pancreatic enzymes and experimental studies supports the suggestion of Wolbach that the pathogenesis of this pancreatic lesion resides in the production of an abnormal pancreatic secretion. Inspissation of this material causes obstruction which leads to dilatation of ducts with connective tissue replacement of atrophied parenchyma, and to pancreatic achylia. The cause for this suggested disturbance to the secretory mechanism in the pancreatic acini remains to be demonstrated.

THE STOMACH IN PERNICIOUS ANEMIA. Alvin J. Cox, San Francisco, Calif.

Abstract. In six autopsied cases of pernicious anemia of different duration and treated for various lengths of time the stomachs showed relatively little postmortem alteration and could be studied thoroughly after being stretched flat and fixed. All showed extensive mucosal changes in the body and fundus like those described by Meulengracht and others in cases of pernicious anemia. The changes were sharply delimited from the pyloric zone and could be distinguished from those in other types of so-called "gastritis." The character of the mucosal change suggests a specific injury of the specific secretory cells of the body and fundus of the stomach with repair by less differentiated epithelial cell types. There is no evidence to suggest a return to normal in the cases which had received prolonged therapy. A case of long-standing sprue with severe macrocytic anemia showed no significant lesions. It is concluded that the gastric changes are not the result of anemia and are not necessarily associated with this type of anemia; but the high incidence of characteristically altered gastric mucosa in patients with pernicious anemia suggests a true relationship which, in the light of recent clinical experimentation, may be etiological. Other examples without gastric lesions may represent similar anemias resulting from different causal factors.